Genes and Immunity (2013) **14,** 286–290

www.nature.com/gene

ORIGINAL ARTICLE

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

V Suppiah[1,2,18], NJ Armstrong[3,4], KS O'Connor[2], T Berg[5,6], M Weltman[7], ML Abate[8], U Spengler[9], M Bassendine[10], GJ Dore[11,12], WL Irving[13], E Powell[14,15], J Nattermann[9], T Mueller[5,6], S Riordan[16], GJ Stewart[2], J George[1], DR Booth[2] and G Ahlenstiel[1] and the International Hepatitis C Genetics Consortium (IHCGC)[17]

IL28B polymorphisms strongly predict spontaneous and treatment-induced clearance of hepatitis C virus (HCV) infection. A recent study proposed a 32-base pair deletion in the CC-chemokine receptor 5 (*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). A cross-sectional cohort of 813 Caucasian patients with CHC genotype 1 (365 responders and 448 non-responders) who had received standard of care dual therapy with interferon (IFN)-α and ribavirin (RBV) was genotyped for the CCR5-Δ32 and *IL28B* polymorphisms to examine their interaction with respect to treatment response. CCR5-Δ32 did not influence treatment-induced recovery to IFN-α/RBV in CHC, and did not improve prediction of sustained virological response 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 European cohorts (2.9% vs 0.4%, $P<0.0001$), but not in Australians of European ancestry. In conclusion, CCR5-Δ32 does not influence treatment response in the context of *IL28B* polymorphisms. Although CCR5-Δ32 may affect viral clearance within closely controlled geographical and genetic environments, we found no effect in larger cohorts treated with dual therapy.

Genes and Immunity (2013) **14,** 286–290; doi:10.1038/gene.2013.15; published online 18 April 2013

Keywords: 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 Hoofnagle[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 1α), CCL4 (macrophagic inflammatory protein 1β), CCL5 (regulated upon activation, normal T cell expressed and secreted) and their receptor, CC-chemokine receptor 5 (CCR5), suggesting a T helper cell type 1-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.*[5] showed that the expression of full-length HCV led to the induction of both *CCR5* messenger RNA and protein in HeLa, Huh7 and HepG2 cell lines. 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 coreceptor for human immunodeficiency virus type 1.[8] A 32-bp deletion in exon 4 of *CCR5* (CCR5-Δ32) leads to truncation and loss of function of the receptor.[9] Homozygosity for this deletion confers high-level (but not complete) resistance to human immunodeficiency virus type 1 infection, whereas heterozygotes show delayed progression to acquired immune deficiency syndrome.[10,11] An increased frequency of CCR5-Δ32 homozygosity was observed in HCV-infected individuals compared with healthy controls (7.8% vs 1.0%).[12] In a later Irish study, the authors found that CCR5-Δ32 was significantly associated with increased spontaneous clearance.[13] Recently, Nattermann *et al.*[14] have shown, in a very well-defined patient cohort ($n=396$) infected with HCV genotype 1 from a

[1]Storr Liver Unit, Westmead Millennium Institute, University of Sydney, Sydney, NSW, Australia; [2]Institute for Immunology and Allergy Research, Westmead Millennium Institute, University of Sydney, Sydney, NSW, Australia; [3]Cancer Research Program, Garvan Institute for Medical Research, University of New South Wales, Sydney, NSW, Australia; [4]School of Mathematics and Statistics, University of New South Wales, Sydney, NSW, Australia; [5]Medizinische Klinik m.S. Hepatologie und Gastroenterologie, Charité, Campus Virchow-Klinikum, Universitätsmedizin, 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, NSW, 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, UK; [11]The Kirby Institute, University of New South Wales, Sydney, NSW, Australia; [12]Department of Immunology and Infectious Diseases, St Vincent's Hospital, Sydney, NSW, Australia; [13]NIHR Biomedical Research Unit in Gastroenterology and the Liver, University of Nottingham, Nottingham, UK; [14]Department of Gastroenterology and Hepatology, Princess Alexandra Hospital, Brisbane, QLD, Australia; [15]The University of Queensland, School of Medicine, Princess Alexandra Hospital, Brisbane, QLD, Australia and [16]Gastrointestinal and Liver Unit, Prince of Wales Hospital and University of New South Wales, Sydney, NSW, Australia. Correspondence: Professor J George, Storr Liver Unit, Westmead Hospital, Hawkesbury Road, Westmead, NSW 2145, Australia.
E-mail: jacob.george@sydney.edu.au
[17]The members of the IHCGC members who participated are listed at the end of the article.
[18]Current address: School of Pharmacy and Medical Sciences, University of South Australia, Adelaide, Australia.
Received 17 December 2012; revised 6 March 2013; accepted 6 March 2013; published online 18 April 2013

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.[15,16] As *IL28B* polymorphisms are currently the strongest host genetic markers to predict treatment-induced clearance of HCV infection,[17–20] 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 with healthy controls, patients with chronic HCV infection had a significantly increased frequency of CCR5-Δ32 homozygosity (Table 1; $P = 0.006$). The association was due to the European cohort, with an increase in CCR5-Δ32 homozygous subjects (2.9% vs 0.4%; $P = 0.0006$) and a decrease in heterozygous subjects (11.3% vs 18.0%; $P = 0.0015$). Further, the enrichment of CCR5-Δ32 homozygous European Caucasian subjects with chronic hepatitis C (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 (interferon (IFN)-α/ribavirin (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 whether the combination of the CCR5-Δ32 deletion with *IL28B* SNPs, *rs12979860* and *rs8099917*, improves prediction of treatment-induced HCV clearance, we used logistic regression modelling, as previously described.[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).

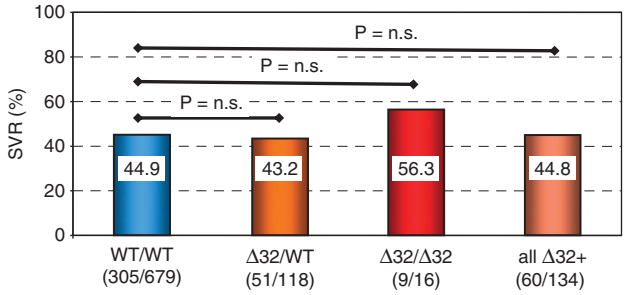

Figure 1. Treatment response based on CCR5-Δ32 status. Response in different CCR5-Δ32 genotypes .WT/WT, homozygous wildtype; Δ32/WT, CCR5-Δ32 heterozygous; Δ32/Δ32, CCR5-Δ32 homozygous; all Δ32 +, all patients carrying one or two CCR5-Δ32 alleles.

Table 1. Genotype distribution of the CCR5-Δ32 among healthy controls and chronic HCV patients

CCR5	*All patients*		*Australian patients*		*European patients*	
	Healthy controls (n = 836)	*Chronic HCV (n = 813)*	*Healthy controls (n = 168)*	*Chronic HCV (n = 300)*	*Healthy controls (n = 668)*	*Chronic HCV (n = 513)*
Genotype						
WT/WT	682 (81.6)	679 (83.5)	137 (82.0)	239 (79.7)	545 (81.6)	440 (85.8)
Δ32/WT	150 (17.9)	118 (14.5)	30 (18.0)	60 (20.0)	120 (18.0)	58 (11.3)
Δ32/Δ32	4 (0.5)	16 (2.0)	1 (1.0)	1 (0.3)	3 (0.4)	15 (2.9)
P-value	0.0047		0.79		$P < 0.0001$	
Allele						
WT	1514 (90.6)	1476 (90.8)	304 (90.5)	538 (89.7)	1210 (90.6)	938 (91.4)
Δ32	158 (9.4)	150 (9.2)	32 (10.7)	62 (10.3)	126 (9.4)	88 (8.6)
P-value	0.82		0.69		0.48	

Abbreviations: CCR5, CC-chemokine receptor 5; HCV, hepatitis C virus; WT, wildtype. Numbers in brackets refer to %.

Table 2. Genotype distribution of the CCR5-Δ32 among responders and non-responders

CCR5	*All patients*		*Australian patients*		*European patients*	
	SVR (n = 365)	*NSVR (n = 448)*	*SVR (n = 129)*	*NSVR (n = 171)*	*SVR (n = 236)*	*NSVR (n = 277)*
Genotype						
WT/WT	305 (83.6)	374 (83.5)	104 (80.6)	135 (78.9)	201 (85.2)	239 (86.3)
Δ32/WT	51 (14.0)	67 (15.0)	25 (19.4)	35 (20.5)	26 (11.0)	32 (11.6)
Δ32/Δ32	9 (2.4)	7 (1.5)	0	1 (0.6)	9 (3.8)	6 (2.2)
P-value	0.62		0.93		0.54	
Allele						
WT	661 (90.5)	815 (91.0)	233 (90.3)	305 (89.2)	428 (90.7)	510 (92.1)
Δ32	69 (9.5)	81 (9.0)	25 (9.7)	37 (10.8)	44 (9.3)	44 (7.9)
P-value	0.78		0.65		0.43	

Abbreviations: CCR5, CC-chemokine receptor 5; NSVR, non-SVR; SVR, sustained virological responders, WT, wildtype. Numbers in brackets refer to %.

Table 3. Breakdown of allelic combinations of two *IL28B* SNPs (*rs8099917* and *rs12979860*) with CCR5-Δ32 mutation in responders and non-responders

CCR5 + IL28B rs8099917	*Total patients* (n = *710*)			*Australian patients* (n = *238*)[a]			*European patients* (n = *472*)		
	Responders (n = *318*)	*Non-responders* (n = *392*)	P-*value*	*Responders* (n = *101*)	*Non-responders* (n = *137*)	P-*value*	*Responders* (n = *217*)	*Non-responders* (n = *255*)	P-*value*
WT/WT + T/T	161 (50.6)	141 (36.0)	<0.0001	58 (57.4)	40 (29.2)	<0.0001	103 (47.5)	101 (39.6)	0.09
WT/WT + T/G	96 (30.2)	162 (41.3)	0.0022	22 (21.8)	59 (43.1)	0.0008	74 (34.1)	103 (40.4)	0.18
WT/WT + G/G	12 (3.8)	25 (6.4)	0.13	2 (2.0)	7 (5.1)	1.00	10 (4.6)	18 (7.1)	0.33
Δ32/WT + T/T	26 (8.2)	23 (5.9)	0.24	12 (11.9)	7 (5.1)	0.09	14 (6.5)	16 (6.3)	1.00
Δ32/WT + T/G	13 (4.1)	36 (9.2)	0.0077	7 (6.9)	24 (17.5)	0.0190	6 (2.8)	12 (4.7)	0.34
Δ32/WT + G/G	1 (0.3)	0	—	0	0	—	1 (0.5)	0	—
Δ32/Δ32 + T/T	8 (2.5)	1 (0.3)	0.0130	0	0	—	8 (3.7)	1 (0.4)	0.0137
Δ32/Δ32 + T/G	1 (0.3)	4 (1.0)	0.39	0	0	—	1 (0.5)	4 (1.6)	—
Δ32/Δ32 + G/G	0	0	—	0	0	—	0	0	—
	Total patients (n = *729*)			*Australian patients* (n = *236*)[b]			*European patients* (n = *493*)		
CCR5 + IL28B rs12979860	*Responders* (n = *328*)	*Non-responders* (n = *401*)	P-*value*	*Responders* (n = *101*)	*Non-responders* (n = *135*)	P-*value*	*Responders* (n = *227*)	*Non-responders* (n = *266*)	P-*value*
WT/WT + C/C	132 (40.2)	68 (17.0)	<0.0001	45 (44.6)	21 (15.6)	<0.0001	87 (38.3)	47 (20.7)	<0.0001
WT/WT + C/T	118 (36.0)	204 (50.9)	<0.0001	32 (31.7)	68 (50.4)	0.0051	86 (37.9)	136 (59.9)	0.0032
WT/WT + T/T	25 (7.6)	65 (16.2)	0.0007	5 (5.0)	16 (11.9)	0.10	20 (8.8)	49 (21.6)	0.0026
Δ32/WT + C/C	22 (6.7)	13 (3.2)	0.0294	8 (7.9)	5 (3.7)	0.25	14 (6.2)	8 (3.5)	0.0022
Δ32/WT + C/T	18 (5.5)	40 (10.0)	0.0259	10 (9.9)	22 (16.3)	0.18	8 (3.5)	18 (7.9)	0.16
Δ32/WT + T/T	4 (1.2)	6 (1.5)	1.00	1 (1.0)	3 (2.2)	0.64	3 (1.3)	3 (1.3)	1.00
Δ32/Δ32 + C/C	3 (0.9)	1 (0.2)	0.33	0	0	—	3 (1.3)	1 (0.4)	0.34
Δ32/Δ32 + C/T	6 (1.8)	3 (0.7)	0.31	0	0	—	6 (2.6)	3 (1.3)	0.31
Δ32/Δ32 + T/T	0	1 (0.2)	—	0	0	—	0	1 (0.4)	—

Abbreviations: CCR5, CC-chemokine receptor 5; SNPs, single-nucleotide polymorphism; WT, wildtype. [a]Total number of Australian and European patients with data for the combination of CCR5 + *IL28B rs809917* genotypes is 238 and 472, respectively. [b]Total number of Australian and European patients with data for the combination of CCR5 + *IL28B rs12979860* genotypes is 236 and 493, respectively.

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, similar to our published data,[12] there was a statistical difference in the CCR5-Δ32 polymorphism frequency between healthy controls and chronically infected patients, probably because of patient heterogeneity and variability in co-exposure to HIV in the different populations. However, it is clear that that CCR5-Δ32 (either on its own or in combination with *IL28B*) does not influence treatment outcome from dual therapy. 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 geographically and socioeconomically analogous backgrounds.[13,14] This suggests that a weak (compared with *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 preselection 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[31] and one of the IFN-induced genes is *CCR5*.[32] *CCR5* is expressed on T helper cell 1 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*.[4] 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.[21] RBV, however, has been postulated to exploit a novel innate mechanism to potentiate the antiviral effects of

IFN-α,[33] which may also involve a bias favouring the T helper cell 1 immune response.[34] 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.[17–20] We cannot exclude the possibility that an influence of the CCR5-Δ32 polymorphism with a small effect size was not discerned, given the cohort size of our study. However, such an effect is likely to have minimal clinical impact, as our NSVR/SVR population size had 90% chance to detect effect sizes of odds ratio of 2.0.

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 host genetics on treatment response.[35] In view of the fact that CCR5 inhibitors are now 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 coinfection. Indeed, a recent study in HIV/HCV-coinfected patients reported no significant changes in viral titres or liver function tests during a short-term course of a CCR5 inhibitor.[36]

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 in Table 4. Briefly, all treated patients were Caucasian and infected with genotype 1, the majority of these patients received pegylated IFN and RBV, and had virological response determined 6 months after completion of therapy. The diagnosis of CHC 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 coinfected 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.[37–40]

Genotyping

The CCR5 Δ32 deletion was genotyped using high resolution melt, as described previously.[41] *IL28B* data presented in this study have been previously published.[16]

Table 4. Comparison of demographic characteristics of the Australian and European patient cohorts

Demographic factors[a]	*Australian patients (n = 300)*	*European patients (n = 513)*
Age (years)	42.6 (8.7)	43.9 (11.1)
Gender (%)[b]		
Females	93 (31.0)	206 (40.2)
Males	207 (69.0)	307 (59.8)
Response status (%)		
Responders	129 (43.0)	236 (46.0)
Non-responders	171 (57.0)	277 (54.0)
BMI ($kg\,m^{-2}$)	27.2 (5.2)	25.4 (4.5)

Abbreviation: BMI, body mass index. [a]Unless otherwise specified, mean (s.d.) are presented. [b]$P<0.05$ comparisons between Australian and European patients based on the χ^2 test.

Statistical analysis

The χ^2 test or Fisher's test where appropriate were used to examine differences in gender, genotype and allele frequencies between SVR vs NSVR, and CHC (that is, NSVR plus SVR) vs healthy controls. The Mann-Whitney test was used to compare the age and BMI between the two groups of patients. P-values <0.05 (two-sided) were regarded as significant. The relationships between CCR5-Δ32 and *IL28B* SNPs, *rs8099917* and *rs12979860*, were investigated using logistic regression. Significance of all models was assessed by likelihood ratio tests. Analysis was carried out in R software (http://www.r-project.org; v2.12).

CONTRIBUTORS

Monika Michalk (University of Bonn, Germany), Barbara Malik (Universitaetsmedizin Berlin, Germany), Patrick McClure (University of Nottingham, UK), Sherie Smith (University of Nottingham, UK), David Sheridan (Newcastle University, UK), Elizabeth Snape (Nepean Hospital, Australia), Vincenzo Fragomeli (Nepean Hospital, Australia), Richard Norris (St Vincent's Hospital, Australia), Dianne How-Chow (St Vincent's Hospital, Australia), Julie R. Jonsson (Princess Alexandra Hospital, Australia), Helen Barrie (Princess Alexandra Hospital, Australia), Sacha Stelzer-Braid (Prince of Wales Hospital, Australia), Shona Fletcher (Prince of Wales Hospital, Australia), Tanya Applegate (National Centre in HIV Epidemiology and Clinical Research), Jason Grebely (National Centre in HIV Epidemiology and Clinical Research), Gail Matthews (National Centre in HIV Epidemiology and Clinical Research), Mandvi Bharadwaj (Burnet Institute, Australia), Antonina Smedile (University of Turin, Italy).

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGEMENTS

This work was supported by research grants from the National Health and Medical Research Council of Australia and by an Australian ARC linkage grant. JG is supported by the Robert W Storr bequest to the University of Sydney Medical Foundation. We thank all patients for their valuable participation in this study. We also thank Reynold Leung for technical assistance.

REFERENCES

1 WHO. Hepatitis C. Fact Sheet No. 164. Revised October 2000. http://www.who.int/mediacentre/factsheets/fs164/en/.
2 Hoofnagle JH. Course and outcome of hepatitis C. *Hepatology* 2002; **36**: S21–S29.
3 Lambotin M, Raghuraman S, Stoll-Keller F, Baumert TF, Barth H. A look behind closed doors: interaction of persistent viruses with dendritic cells. *Nat Rev Microbiol* 2010; **8**: 350–360.
4 Shields PL, Morland CM, Salmon M, Qin S, Hubscher SG, Adams DH. Chemokine and chemokine receptor interactions provide a mechanism for selective T cell recruitment to specific liver compartments within hepatitis C-infected liver. *J Immunol* 1999; **163**: 6236–6243.
5 Soo HM, Garzino-Demo A, Hong W, Tan YH, Tan YJ, Goh PY *et al.* Expression of a full-length hepatitis C virus cDNA up-regulates the expression of CC chemokines MCP-1 and RANTES. *Virology* 2002; **303**: 253–277.
6 Nattermann J, Nischalke HD, Feldmann G, Ahlenstiel G, Sauerbruch T, Spengler U. Binding of HCV E2 to CD81 induces RANTES secretion and internalization of CC chemokine receptor 5. *J Viral Hepat* 2004; **11**: 519–526.
7 Lichterfeld M, Leifeld L, Nischalke HD, Rockstroh JK, Hess L, Sauerbruch T *et al.* Reduced CC chemokine receptor (CCR) 1 and CCR5 surface expression on peripheral blood T lymphocytes from patients with chronic hepatitis C infection. *J Infect Dis* 2002; **185**: 1803–1807.
8 Deng H, Liu R, Ellmeier W, Choe S, Unutmaz D, Burkhart M *et al.* Identification of a major co-receptor for primary isolates of HIV-1. *Nature* 1996; **381**: 661–666.
9 Samson M, Libert F, Doranz BJ, Rucker J, Liesnard C, Farber CM *et al.* Resistance to HIV-1 infection in caucasian individuals bearing mutant alleles of the CCR-5 chemokine receptor gene. *Nature* 1996; **382**: 722–725.
10 Dean M, Carrington M, Winkler C, Huttley GA, Smith MW, Allikmets R *et al.* Genetic restriction of HIV-1 infection and progression to AIDS by a deletion allele of the CKR5 structural gene. Hemophilia Growth and Development Study, Multicenter

AIDS Cohort Study, Multicenter Hemophilia Cohort Study, San Francisco City Cohort, ALIVE Study. *Science* 1996; **273**: 1856–1862.
11 Stewart G. Chemokine genes--beating the odds. *Nat Med* 1998; **4**: 275–257.
12 Woitas RP, Ahlenstiel G, Iwan A, Rockstroh JK, Brackmann HH, Kupfer B *et al.* Frequency of the HIV-protective CC chemokine receptor 5-Delta32/Delta32 genotype is increased in hepatitis C. *Gastroenterology* 2002; **122**: 1721–1728.
13 Goulding C, McManus R, Murphy A, MacDonald G, Barrett S, Crowe J *et al.* The CCR5-delta32 mutation: impact on disease outcome in individuals with hepatitis C infection from a single source. *Gut* 2005; **54**: 1157–1161.
14 Nattermann J, Timm J, Nischalke HD, Olbrich A, Michalk M, Tillmann HL *et al.* The predictive value of IL28B gene polymorphism for spontaneous clearance in a single source outbreak cohort is limited in patients carrying the CCR5Delta32 mutation. *J Hepatol* 2011; **55**: 1201–1206.
15 Thomas DL, Thio CL, Martin MP, Qi Y, Ge D, O'Huigin C *et al.* Genetic variation in IL28B and spontaneous clearance of hepatitis C virus. *Nature* 2009; **461**: 798–801.
16 Suppiah V, Gaudieri S, Armstrong NJ, O'Connor KS, Berg T, Weltman M *et al.* IL28B, HLA-C, and KIR variants additively predict response to therapy in chronic hepatitis C virus infection in a European cohort: a cross-sectional study. *PLoS Med* 2011; **8**: e1001092.
17 Ge D, Fellay J, Thompson AJ, Simon JS, Shianna KV, Urban TJ *et al.* Genetic variation in IL28B predicts hepatitis C treatment-induced viral clearance. *Nature* 2009; **461**: 399–401.
18 Suppiah V, Moldovan M, Ahlenstiel G, Berg T, Weltman M, Abate ML *et al.* IL28B is associated with response to chronic hepatitis C interferon-alpha and ribavirin therapy. *Nat Genet* 2009; **41**: 1100–1104.
19 Tanaka Y, Nishida N, Sugiyama M, Kurosaki M, Matsuura K, Sakamoto N *et al.* Genome-wide association of IL28B with response to pegylated interferon-alpha and ribavirin therapy for chronic hepatitis C. *Nat Genet* 2009; **41**: 1105–1109.
20 Rauch A, Kutalik Z, Descombes P, Cai T, Di Iulio J, Mueller T *et al.* Genetic variation in IL28B is associated with chronic hepatitis C and treatment failure: a genome-wide association study. *Gastroenterology* 2010; **138**: 1338–1345.
21 Ahlenstiel G, Berg T, Woitas RP, Grunhage F, Iwan A, Hess L *et al.* Effects of the CCR5-Delta32 mutation on antiviral treatment in chronic hepatitis C. *J Hepatol* 2003; **39**: 245–252.
22 Promrat K, McDermott DH, Gonzalez CM, Kleiner DE, Koziol DE, Lessie M *et al.* Associations of chemokine system polymorphisms with clinical outcomes and treatment responses of chronic hepatitis C. *Gastroenterology* 2003; **124**: 352–360.
23 Hellier S, Frodsham AJ, Hennig BJ, Klenerman P, Knapp S, Ramaley P *et al.* Association of genetic variants of the chemokine receptor CCR5 and its ligands, RANTES and MCP-2, with outcome of HCV infection. *Hepatology* 2003; **38**: 1468–1476.
24 Mascheretti S, Hinrichsen H, Ross S, Buggisch P, Hampe J, Foelsch UR *et al.* Genetic variants in the CCR gene cluster and spontaneous viral elimination in hepatitis C-infected patients. *Clin Exp Immunol* 2004; **136**: 328–333.
25 Wasmuth HE, Werth A, Mueller T, Berg T, Dietrich CG, Geier A *et al.* CC chemokine receptor 5 delta32 polymorphism in two independent cohorts of hepatitis C virus infected patients without hemophilia. *J Mol Med* 2004; **82**: 64–69.
26 Goyal A, Suneetha PV, Kumar GT, Shukla DK, Arora N, Sarin SK. CCR5Delta32 mutation does not influence the susceptibility to HCV infection, severity of liver disease and response to therapy in patients with chronic hepatitis C. *World J Gastroenterol* 2006; **12**: 4721–4726.
27 Glas J, Torok HP, Simperl C, Konig A, Martin K, Schmidt F *et al.* The delta 32 mutation of the chemokine-receptor 5 gene neither is correlated with chronic hepatitis C nor does it predict response to therapy with interferon-alpha and ribavirin. *Clin Immunol* 2003; **108**: 46–50.
28 Fried MW, Shiffman ML, Reddy KR, Smith C, Marinos G, Goncales Jr FL *et al.* Peginterferon alfa-2a plus ribavirin for chronic hepatitis C virus infection. *N Engl J Med* 2002; **347**: 975–982.
29 Hadziyannis SJ, Sette Jr H, Morgan TR, Balan V, Diago M, Marcellin P *et al.* Peginterferon-alpha2a and ribavirin combination therapy in chronic hepatitis C: a randomized study of treatment duration and ribavirin dose. *Ann Intern Med* 2004; **140**: 346–355.
30 Novembre J, Galvani AP, Slatkin M. The geographic spread of the CCR5 Delta32 HIV-resistance allele. *PLoS Biol* 2005; **3**: e339.
31 Shin EC, Seifert U, Kato T, Rice CM, Feinstone SM, Kloetzel PM *et al.* Virus-induced type I IFN stimulates generation of immunoproteasomes at the site of infection. *J Clin Invest* 2006; **116**: 3006–3014.
32 Yang YF, Tomura M, Iwasaki M, Ono S, Zou JP, Uno K *et al.* IFN-alpha acts on T-cell receptor-triggered human peripheral leukocytes to up-regulate CCR5 expression on CD4+ and CD8+ T cells. *J Clin Immunol* 2001; **21**: 402–429.
33 Thomas E, Feld JJ, Li Q, Hu Z, Fried MW, Liang TJ. Ribavirin potentiates interferon action by augmenting interferon-stimulated gene induction in hepatitis C virus cell culture models. *Hepatology* 2001; **53**: 32–41.
34 Tam RC, Pai B, Bard J, Lim C, Averett DR, Phan U *et al.* Ribavirin polarizes human T cell responses towards a Type 1 cytokine profile. *J Hepatol* 1999; **30**: 376–382.
35 Ahlenstiel G, Booth DR, Goerge J. Will IL28B polymorphism remain relevant to DAA treatment paradigms? *Antiviral Therapy* 2012; **17**(6 Pt B): 1163–1170.
36 Fatkenheuer G, Hoffmann C, Slim J, Rouzier R, Keung A, Li J *et al.* Short-term administration of the CCR5 antagonist vicriviroc to patients with HIV and HCV coinfection is safe and tolerable. *J Acquir Immune Defic Syndr* 2010; **53**: 78–85.
37 Bennetts BH, Teutsch SM, Buhler MM, Heard RN, Stewart GJ. The CCR5 deletion mutation fails to protect against multiple sclerosis. *Hum Immunol* 1997; **58**: 52–59.
38 Libert F, Cochaux P, Beckman G, Samson M, Aksenova M, Cao A *et al.* The deltaccr5 mutation conferring protection against HIV-1 in Caucasian populations has a single and recent origin in Northeastern Europe. *Hum Mol Genet* 1998; **7**: 399–406.
39 Spagnolo P, Renzoni EA, Wells AU, Copley SJ, Desai SR, Sato H *et al.* C-C chemokine receptor 5 gene variants in relation to lung disease in sarcoidosis. *Am J Respir Crit Care Med* 2005; **172**: 721–728.
40 Oh DY, Jessen H, Kucherer C, Neumann K, Oh N, Poggensee G *et al.* CCR5Delta32 genotypes in a German HIV-1 seroconverter cohort and report of HIV-1 infection in a CCR5Delta32 homozygous individual. *PLoS One* 2008; **3**: e2747.
41 Nischalke HD, Nattermann J, Lichterfeld M, Woitas RP, Rockstroh JK, Sauerbruch T *et al.* Rapid determination of the Delta32 deletion in the human CC-chemokine receptor 5 (CCR5) gene without DNA extraction by lightcycler real-time polymerase chain reaction. *AIDS Res Hum Retroviruses* 2004; **20**: 750–754.