CLINICAL ADVANCES IN LIVER, PANCREAS, AND BILIARY TRACT

Genetic Variation in *IL28B* Is Associated With Chronic Hepatitis C and Treatment Failure: A Genome-Wide Association Study

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BACKGROUND & AIMS: Hepatitis C virus (HCV) induces chronic infection in 50% to 80% of infected persons; approximately 50% of these do not respond to therapy. We performed a genome-wide association study to screen for host genetic determinants of HCV persistence and response to therapy. **METHODS:** The analysis included 1362 individuals: 1015 with chronic hepatitis C and 347 who spontaneously cleared the virus (448 were coinfected with human immunodeficiency virus [HIV]). Responses to pegylated interferon alfa and ribavirin were assessed in 465 individuals. Associations between more than 500,000 single nucleotide polymorphisms (SNPs) and outcomes were assessed by multivariate logistic regression. **RESULTS:** Chronic hepatitis C was associated with SNPs in the *IL28B* locus, which encodes the antiviral cytokine interferon lambda. The *rs8099917* minor allele was associated with progression to chronic HCV infection (odds ratio [OR], 2.31; 95% confidence interval [CI], 1.74–3.06; $P = 6.07 \times 10^{-9}$). The association was observed in HCV mono-infected (OR, 2.49; 95% CI, 1.64–3.79; $P = 1.96 \times 10^{-5}$) and HCV/HIV coinfected individuals (OR, 2.16; 95% CI, 1.47–3.18; $P = 8.24 \times 10^{-5}$). *rs8099917* was also associated with failure to respond to therapy (OR, 5.19; 95% CI, 2.90–9.30; $P = 3.11 \times 10^{-8}$), with the strongest effects in patients with HCV genotype 1 or 4. This risk allele was identified in 24% of individuals with spontaneous HCV clearance, 32% of chronically infected patients who responded to therapy, and 58% who did not respond ($P = 3.2 \times 10^{-10}$). Resequencing of *IL28B* identified distinct haplotypes that were associated with the clinical phenotype. **CONCLUSIONS: The association of the *IL28B* locus with natural and treatment-associated control of HCV indicates the importance of innate immunity and interferon lambda in the pathogenesis of HCV infection.**

Keywords: Hepatitis C; Genetics; Interferon; Interleukin-28.

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Hepatitis C virus (HCV) is a positive-stranded RNA virus that chronically infects 120 to 180 million people (ie, ~3% of the world population).[1] Infection with HCV induces a wide range of innate and adaptive immune responses that achieve permanent control of HCV in 20% to 50% of infected individuals.[2,3] Failure to clear

Abbreviations used in this paper: CI, confidence interval; HIV, human immunodeficiency virus; IFN, interferon; OR, odds ratio; SNP, single nucleotide polymorphism.

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HCV leads to chronic hepatitis C. The morbidity and mortality associated with chronic hepatitis C are mainly attributable to its progression toward cirrhosis and hepatocellular carcinoma.[4] Standard therapy with pegylated interferon (IFN)-α and ribavirin fails to clear HCV in ~50% of chronically infected individuals.[5–8]

Host factors influence both the natural course of hepatitis C and response to therapy.[9–12] In 2 cohorts of pregnant women infected under similar conditions with anti-D immunoglobulin preparations contaminated with a single strain of HCV, half spontaneously cleared the infection and half progressed to chronic hepatitis C.[13,14] Among chronically infected patients, response to treatment differs, even among cases with similar HCV RNA levels and identical viral genotypes.[4–7] The response rates are associated with viral genotypes, ethnicity, and sex.[15–18]

Previous candidate gene studies reported the role of genetic polymorphisms of HLA,[9,12,19] killer immunoglobulin-like receptors,[20] chemokines, and interleukins as well as IFN-stimulated genes[21–25] on spontaneous HCV clearance. A recent candidate gene study showed that genetic variation in the *IL28B* gene, which encodes IFN-λ, is associated with spontaneous HCV clearance,[26] and 3 genome-wide association studies reported associations of SNPs in *IL28B* with response to antiviral therapy[27–29] in individuals infected with HCV genotype 1.

In this study, we systematically searched for common human genetic determinants of progression to chronic hepatitis C and of treatment failure using a genome-wide association study in 1362 HCV-infected individuals.

Patients and Methods

Patients were included from the Swiss Hepatitis C Cohort Study and the Swiss HIV Cohort Study, 2 multicenter studies performed at 8 major Swiss hospitals and their local affiliated centers,[30,31] and from the Medical Clinic for Hepatology and Gastroenterology, Medical University Charité Campus, Virchow–Klinikum Berlin, in Berlin, Germany. Written informed consent, including genetic testing, was mandatory for inclusion, and the study was approved by all local ethical committees. Due to the different genetic predictors of hepatitis C outcomes in racially diverse populations,[15,18] analyses were limited to the white population. Demographic characteristics including age, sex, HCV risk factors, HCV genotypes, alcohol consumption, markers for hepatitis B virus and human immunodeficiency virus (HIV) infection, HCV viral load, liver biopsy data, and HCV treatment were extracted from clinical databases.

Chronic HCV infection was defined as anti-HCV seropositivity (using enzyme-linked immunosorbent assay and confirmed by immunoblot or recombinant immunoblot assay) and detectable HCV RNA by quantitative or qualitative assays; spontaneous HCV clearance was defined as HCV seropositivity and undetectable HCV RNA in patients without previous antiviral treatment. To avoid the fluctuations of HCV RNA levels during the first year of infection (reviewed by Hoofnagle[32]), we determined HCV RNA levels at least 1 year after the first documented positive HCV serology. Patients who received at least 80% of the recommended dose of pegylated IFN-α/ribavirin were considered assessable for response to treatment. Sustained viral response was defined as an undetectable HCV RNA in serum more than >24 weeks after treatment termination; all other patients were considered nonresponders. Severe fibrosis was considered in patients with a METAVIR score ≥F3.

Genotyping of more than 500,000 common human polymorphisms was performed by the Genomics Platform of the National Center of Competence in Research "Frontiers in Genetics" at the University of Geneva in Geneva, Switzerland, by using Illumina Human1M-Duo, HumanHap550, or Human610W-Quad BeadChips (Illumina, San Diego, CA). Genotype calling was performed using the default settings of the BeadStudio software (Illumina). Calls with a genotyping score <0.2 were excluded from further analysis. Single nucleotide polymorphisms (SNPs) with a call rate <90% and individuals with a call rate <95% were excluded. To enable multiple platform analysis, genome-wide imputation was performed using MACH[33] based on measured SNPs with >90% call rate, minor allele frequency >1%, and Hardy–Weinberg P value $>10^{-7}$. SNPs with low imputation quality (r2-hat <0.3) were ignored. Whenever the measured genotype was available, it replaced the imputed value. Population stratification and relatedness were assessed using the ancestry principal components as previously described.[34–36] One of each genetically related/identical individual pair (relatedness >0.125) was excluded from further analysis.

To take into account the potential influence of HIV coinfection on spontaneous HCV clearance, HCV monoinfected and coinfected individuals were first analyzed separately; subsequently, we performed a genome-wide meta-analysis of the 2 cohorts. A meta-analysis of association signals obtained from each cohort was performed using inverse variance weighting. Association analysis was performed using a logistic regression model with exact maximum likelihood estimation. Covariates influencing the outcome in the univariate analysis ($P < 0.1$), along with the first 2 ancestry principal components, were included in the model. We used a mild P value cutoff as an inclusion criterion for covariates to avoid disregarding potentially important factors. To account for the fact that different genotyping platforms were used, we excluded any SNP with an allele frequency (among patients with chronic infection) that was significantly different (χ^2 test, $P < 10^{-4}$) between any 2 platforms. Genomic control was applied to the genome-wide P values yielding a λ of 1.04 (for the mono-infected cohort) or 1.02 (for the coinfected cohort). These values suggested very mild inflation and confirmed that possible

population stratification was sufficiently corrected by including the first 2 ancestry principal components in the models. Bonferroni correction was used to adjust for multiple testing; we used 5×10^{-8} as significance threshold.

Resequencing of the candidate locus and recombinant mapping[37] were performed for the purpose of mapping the candidate causal variant or genetic region using the primers indicated in Supplementary Table 1. We used PHASE version 2.1 software (University of Washington, Seattle, WA) for haplotype inference from population genotype data.

Results

Chronic Versus Spontaneously Cleared HCV Infection

The study included 1362 patients with HCV infection, among whom 347 had spontaneously cleared HCV infection and 1015 had progressed to persistent infection; 914 were HCV mono-infected and 448 were coinfected with HIV/HCV (Supplementary Table 2). Several SNPs near the *IL28* locus on chromosome 19 were associated with chronic HCV infection with genome-wide significance (Figure 1). The top hit *rs8099917* (odds ratio [OR], 2.31; 95% confidence interval [CI], 1.74–3.06; $P = 6.07 \times 10^{-9}$; Figure 1) is located in an ~80-kilobase region encoding 3 cytokines (ie, IL28B, IL28A, and IL29) as T (major allele) to G (minor allele) substitution 7554 base pairs upstream the start codon of *IL28B* (Supplementary Figure 1). The signal in the locus carried 7 SNPs with a *P* value $<10^{-5}$ (Supplementary Figure 1*A*). The recombination profile indicated that the signal encompassed the whole *IL28B* gene (Supplementary Figures 1 and 2). The effect was similar in HCV mono-infected (OR, 2.49; 95% CI, 1.64–3.79; $P = 1.96 \times 10^{-5}$) and HCV/HIV coinfected individuals (OR, 2.16; 95% CI, 1.47–3.18; $P = 8.24 \times 10^{-5}$). No SNP outside the *IL28B/A* locus reached genome-wide significance (Figure 1 and Supplementary Tables 3 and 4).

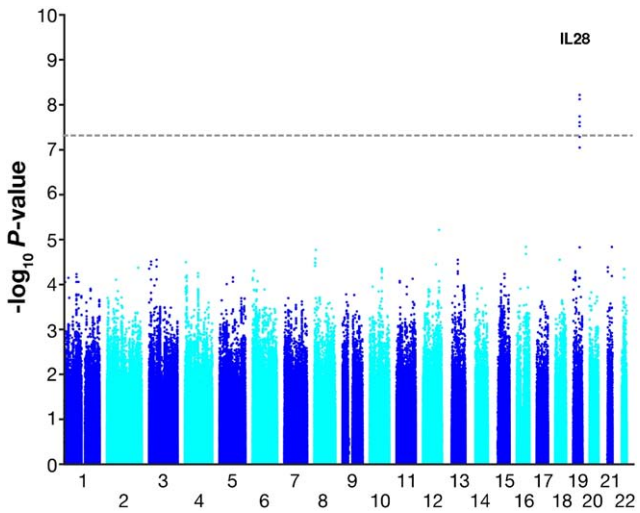

Figure 1. Manhattan plot for chronic versus spontaneously cleared HCV infection. The association values in $-\log_{10} P$ values are shown by chromosome. Genome-wide significance is indicated by the *horizontal line*. A genome-wide significant association signal is observed on chromosome 19. The signal maps to the *IL28* locus. *rs8099917* is the top hit.

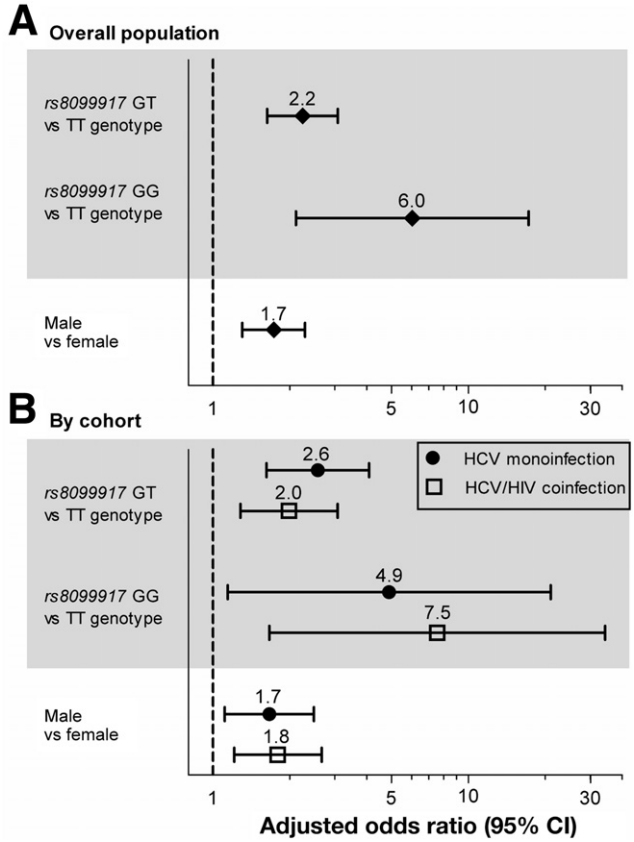

Figure 2. Predictors of chronic versus spontaneously cleared HCV infection. (*A*) Carriers of the rs8099917 G-risk genotypes had a higher risk of failing to spontaneously clear HCV infection and thus to progress to chronic infection. (*B*) The effect of *rs8099917* was similar in HCV mono-infected and HIV/HCV coinfected individuals. ORs were calculated by allele and were adjusted for sex and the first 2 ancestry principal components. Age was not included because the time point of acute HCV infection is unknown in most cases.

The frequencies of the *rs8099917* TT, GT, and GG genotypes were 0.58, 0.37, and 0.05 among patients with chronic infection versus 0.78, 0.21, and 0.01 among those with spontaneous clearance, respectively (Supplementary Table 5). In the analyses by genotype, both homozygous (GG: OR, 6.02; 95% CI, 2.10–17.21; $P = 8.10 \times 10^{-4}$) and heterozygous (GT: OR, 2.24; 95% CI, 1.63–3.07; $P = 6.63 \times 10^{-7}$) patients had a higher risk of chronicity compared with patients carrying the common genotype (TT) (Figure 2). Thus, the minor G allele increased the risk of chronicity and was defined as the risk allele. The effect of the genotypes was very similar for both the HCV mono-infected and HCV/HIV coinfected individuals (Figure 2).

As expected, male sex was associated with higher chronicity rates (OR, 1.67; 95% CI, 1.29–2.16; $P = 9.5 \times 10^{-5}$). The association between sex and HCV persistence was not modified relevantly by adjusting for *rs8099917* (OR, 1.80; 95% CI, 1.41–2.31; $P = 2.77 \times 10^{-6}$), and there was no significant interaction between sex and this SNP ($P > .05$).

Among those with chronic HCV infection, there was no significant association of *rs8099917* with HCV RNA levels (OR, 1.01; 95% CI, 0.81–1.25; $P = .94$).

Treatment Response

Next, we assessed whether the SNPs associated with chronicity also influenced response to pegylated IFN-α/ribavirin combination therapy. Among chronically infected patients, 465 were assessable for response to this treatment (all mono-infected). Factors associated with failure to treatment included HCV genotype 1 or 4 ($P < .001$), severe fibrosis ($P = .06$), male sex ($P = .05$), older age ($P < .01$), and higher pretreatment HCV RNA ($P = .04$). These variables were included as covariates in the logistic regression.

The frequencies of genotypes TT, GT, and GG for the previously discovered *rs8099917* were 0.42, 0.51, and 0.07 among patients with treatment failure versus 0.68, 0.29, and 0.03 among those with sustained viral response, respectively (Supplementary Table 5). In the analyses by genotypes, GG homozygous and GT heterozygous patients both had a higher risk of treatment failure compared with patients carrying the common genotype (TT) (Figure 3). Minor G allele carriers had a higher risk of treatment failure than the other patients (OR, 5.19; 95% CI, 2.90–9.30; $P = 3.11 \times 10^{-8}$), and the G allele was therefore defined as the risk allele.

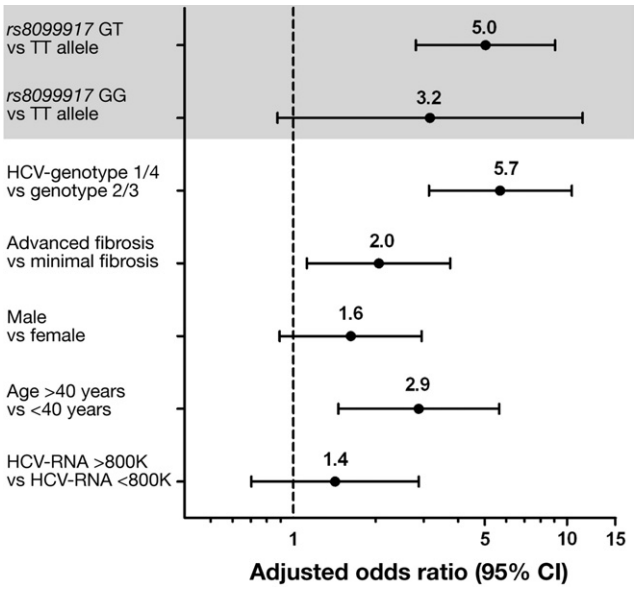

Figure 3. Predictors of failure to respond to pegylated IFN-α and ribavirin therapy. Carriers of the *rs8099917* G-risk genotypes had a higher risk of failing to respond to HCV treatment. ORs were calculated by allele and were adjusted for HCV genotypes, fibrosis stage, sex, age, baseline HCV viral load, and the first 2 ancestry principal components.

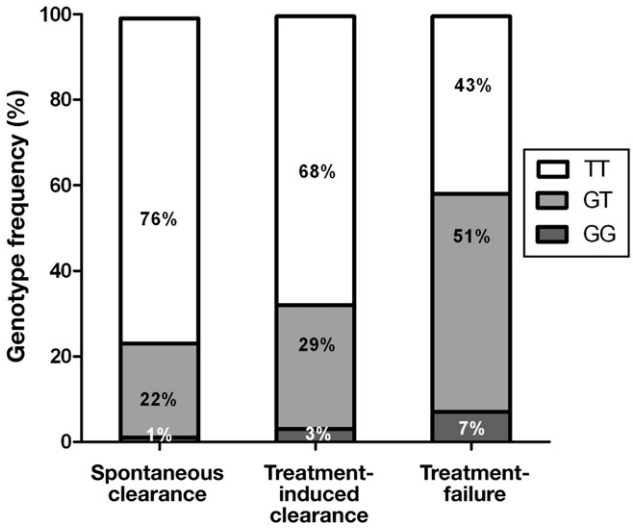

Figure 4. Distribution of genotypes in an infected population. Among 914 mono-infected patients, those with treatment failure have higher rates of carriage of the *rs8099917* G-risk allele than patients with treatment-induced clearance and patients with spontaneous clearance. There was a significant trend ($P = 4.83 \times 10^{-9}$) across the 3 groups.

Clinical Impact

Given that *rs8099917* was associated with both chronicity and treatment failure, we assessed data as a theoretical continuum, assuming the history of an infected population (Figure 4). Overall, the proportion of individuals carrying the risk allele increased progressively from a low frequency among patients with spontaneous clearance (24%) to an intermediate frequency among chronically infected patients with sustained viral response to treatment (32%) and to a highest frequency among chronically infected individuals who failed to respond to treatment (58%; $P = 3.2 \times 10^{-10}$).

A second aspect of importance in clinical care is viral genotype. Therefore, we assessed the joint contribution of host and pathogen genetic risk determinants. Patients were stratified in 4 groups, according to the viral genotypes (viral genotype 2 or 3 vs viral genotype 1 or 4) and host polymorphisms (host *rs8099917* G risk allele carriers vs noncarriers; Table 1). Treatment failure occurred in only 14% of patients with both low-risk parameters compared with 72% among those with both high-risk parameters (OR, 15.79; 95% CI, 8.37–29.76; $P = 1.48E\text{-}17$; Table 1). Among patients infected with genotypes 1 or 4, treatment failure occurred in 72% of risk allele carriers infected compared with only 39% of noncarriers (OR, 4.97; 95% CI, 2.56–9.66; $P = 2.13 \times 10^{-6}$). There was no significant association between genetic variation in *IL28B* and response to therapy among individuals infected with HCV genotype 2 or 3 (OR, 1.58; 95% CI, 0.77–3.25; $P = .18$).

Table 1. Joint Analysis of Viral and Host Genetic Determinants of Treatment Response

HCV genotype	*IL28B rs8099917* G allele	Treatment failure n	Treatment failure Frequency	Treatment success n	Treatment success Frequency	Failure (%)	OR (95% CI)[a]	*P* value
2/3	Absent	20	0.13	127	0.42	14		Referent
2/3	Present	17	0.11	66	0.22	20	1.62 (0.80–3.31)	1.81E-01
1/4	Absent	48	0.30	81	0.27	37	3.95 (2.19–7.12)	4.75E-06
1/4	Present	74	0.47	29	0.10	72	15.79 (8.37–29.76)	1.48E-17

[a]Adjusted for fibrosis stage, sex, age, baseline HCV viral load, and the first 2 ancestry principal components.

Recombinant Mapping

To map the candidate causal variant or genetic region tagged by *rs8099917*, we resequenced the *IL28B* locus. To maximize the likelihood of identifying the causal region, we performed recombinant mapping on DNA from individuals selected for having "concordant" or "discordant" genotype-phenotype constellations. Concordant referred to individuals homozygous for the common allele TT with clearance (n = 15) or homozygous for the *rs8099917* GG risk allele with chronic infection (n = 15). Discordant referred to individuals homozygous for the common allele TT with chronic infection (n = 15) or the rare individuals (n = 2) homozygous for the GG risk allele with HCV clearance.

Resequencing of the *IL28B* locus based on recombinant mapping identified 21 SNPs. Haplotype inference led to the identification of 2 main haplotype families (Figure 5). The first family carried the individuals with an HCV clearance phenotype. The second family of haplotypes carried most of the risk of chronicity. The sequence of the *IL28B* promoter and coding region in discordant individuals that progressed to chronic infection despite absence

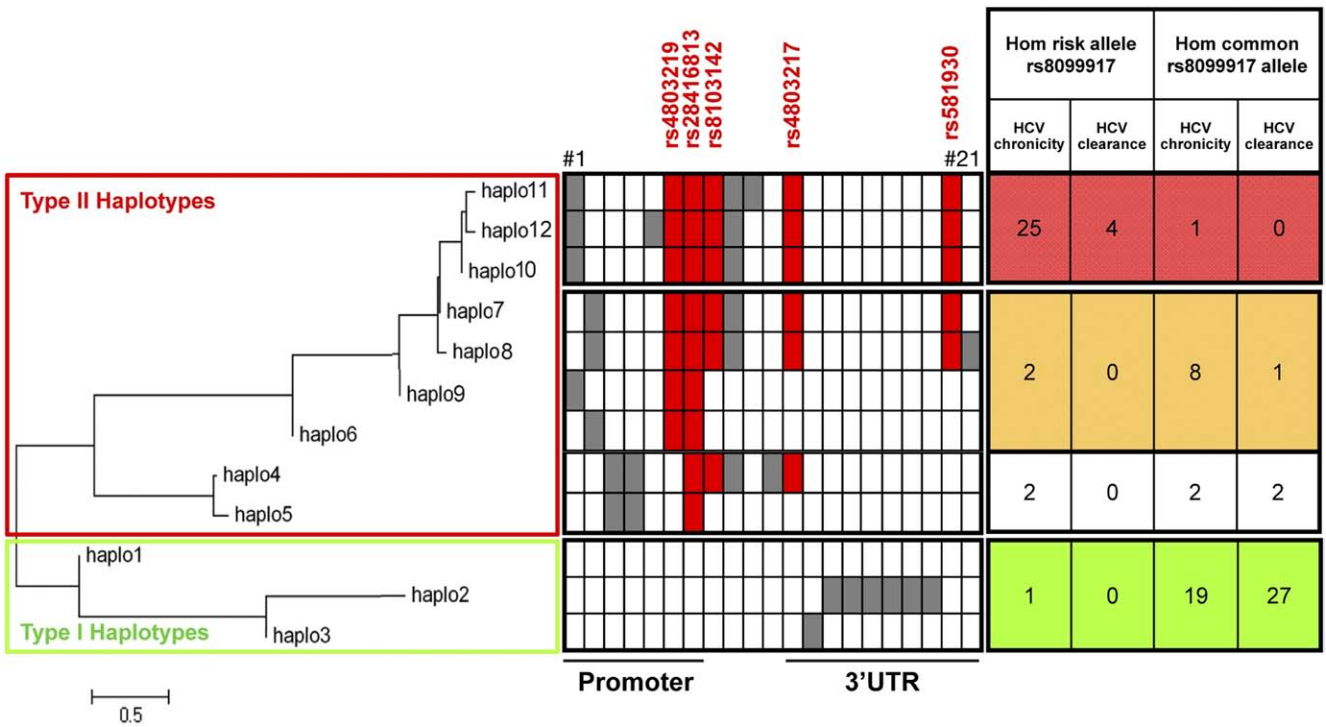

Figure 5. Resequencing, haplotype inference, and recombinant mapping of the candidate causal region in *IL28B*. Resequencing for the purpose of mapping the causal variant or region in *IL28B* was performed by using the primers indicated in Supplementary Table 5 to amplify 4279 base pairs. Sequencing was performed on DNA from 47 individuals representing the various constellations of marker genotype at *rs8099917* and the HCV clearance phenotype. This led to the identification of 21 SNPs (#1 to #21, presence of a polymorphism is indicated by a *shaded box*). Inference of the various haplotypes by using PHASE led to the proposal of a dendrogram with 2 main haplotypic families. The frequent haplotype 1 and derived haplotypes constitute the type I haplotype family, which is generally associated with HCV clearance. The frequent haplotype 10 and derived haplotypes constitute the type II haplotype family, which is generally associated with chronicity. Type II haplotypes are characterized by a defined structure that includes 2 promoter SNPs, a nonsynonymous K70R, and two 3′ untranslated region SNPs (marked *red*). The *numbers* in the *right panel* indicate the number of chromosomes with the respective haplotype – *rs8099917* combinations by clinical outcome.

of the risk allele of *rs8099917* was characteristic of that of individuals with the risk marker. Several specific SNPs were identified as candidates for being causal (Figure 5).

Discussion

This is the first genome-wide association study to report the influence of human genetic variation on the natural control of HCV infection. We found a similar effect of the same genetic marker (*rs8099917*, located near the *IL28B* gene) for both natural and treatment-induced control of HCV infection. The lowest carriage frequency of the risk allele (24%) was observed among persons with spontaneous clearance and increased to 32% among chronically infected patients who responded to treatment and to 58% among chronically infected patients who failed to respond. This observation is in line with recent studies that observed a strong association of genetic variation in *IL28B* with response to therapy[27–29] and with spontaneous HCV clearance.[26]

Our findings strongly point to a major role of the innate immunity in the control of HCV. The mapped region (19q13) encodes 3 cytokine genes (IL28A, IL28B, and IL29) that belong to the IFN-λ (also named type III IFN) family. IFN-λs interact with a transmembrane receptor to induce potent antiviral responses.[38–40] This antiviral activity is mediated through the activation of the JAK-STAT (IFN-αs, IFN-γs, and IFN-λs) and MAPK (IFN-αs and IFN-λs) pathways (reviewed by Li et al[39]). In vitro and in vivo models have shown the importance of IFN-λs in the immune response to several viral pathogens, including herpes simplex virus,[41,42] cytomegalovirus,[43] HIV,[44] and hepatitis B virus.[45] IFN-λ1 and IFN-λ2 blocked HCV replication in human hepatocytic cell lines.[46–48] IFN-λ has been proposed, and already tested, as a treatment of hepatitis C.[49,50] In a phase 1B trial of patients with chronic hepatitis C who are responders/relapsers, the drug had a robust activity against HCV and limited toxicity.[49,51] This low toxicity may be explained by a reduced tissue expression of the IFN-λ receptor compared with that of the IFN-α receptor.[49,50] Thus, IFN-λ is a promising molecule for the future treatment of hepatitis C, and a dose-ranging phase 2 trial is currently planned.

The genetic data from this study point to *IL28B* as a critical effector in the control of HCV. The mechanisms linking *rs8099917* with differential antiviral responses in HCV-infected patients need to be elucidated. The SNP is located 8 kilobases upstream of the start codon of *IL28B*. As part of a haplotype block that covers the full length of *IL28B*, it may interfere with transcription factors and influence gene expression or splicing. In 2 studies,[28,29] the presence of the *rs8099917* risk allele was associated with lower expression of IFN-λ.

Carriage of the *rs8099917* SNP was the strongest genetic predictor of both natural and treatment-induced control of HCV. This SNP was also the strongest predictor for response to HCV therapy in white[27,28] and Japanese[29] populations. Thomas et al reported a strong association of the *rs12979860* SNP with spontaneous HCV clearance.[26] *rs12979860* is not measured on the Illumina 550 chip and was not available in 149 individuals from our cohort who were genotyped with this chip. In the 1213 individuals with available data, carriage of *rs12979860* was highly associated with chronic HCV infection (OR, 1.95; 95% CI, 1.54–2.48; $P = 3.38 \times 10^{-8}$) and was in strong linkage disequilibrium with *rs8099917* ($D' = 0.98$; $r^2 = 0.5$).

SNPs associated with chronic infection may be in linkage disequilibrium with one or several coding SNP(s), or functional noncoding SNP(s), in *IL-28B* that modulate its function. For this purpose, we used recombinant mapping exploiting the various possible couplings of *rs8099917* and the clearance/chronicity phenotype. This led to the identification of 2 main haplotypes (and their derived haplotypes) in the study population. Each haplotypic family had a characteristic promoter and coding region that associates with the clinical phenotype. This constitutes a lead to the causal allele and mechanism of action of *IL28B* in clearance. Functional studies of gene expression and cytokine production among the different allele carriers will help in addressing this issue.

Our study shows that genetic variation in *IL28B* is genome-wide the strongest common human genetic determinant for the control of HCV infection. No SNP outside the *IL28B/A* locus reached genome-wide significance. This further underscores the particular role of innate immune responses for the control of HCV infection. The comparison with genome-wide associations in HIV infection is intriguing, because all significant determinants for the control of HIV were within the major histocompatibility complex on chromosome 6 in genes involved in adaptive immune responses.[52]

Our findings could have a substantial impact for prognosis and therapy. For example, individuals with HCV genotype 1 or 4 who carry the risk allele, particularly in homozygosis, will have a very low probability of natural or treatment-induced clearance. These individuals would be prime candidates for novel therapeutic strategies. However, emergence of HCV variants that are resistant to small molecules is almost inevitable if these drugs are not combined with other effective compounds.[53] Individuals with unfavorable host and viral genotypes treated with pegylated IFN-α/ribavirin and a small molecule would be treated with only one effective drug and therefore at high risk for selection of drug resistance mutations and treatment failure. Future studies need to address the question whether individuals with unfavorable host and viral genotypes would benefit from combining different antiviral molecules to maximize viral response and minimize the risk of drug resistance.

There was no significant association between genetic variation in *IL28B* and response to therapy among indi-

viduals infected with HCV genotype 2 or 3, indicating that the prognostic value of the risk allele for treatment response might be limited to individuals with difficult-to-treat HCV genotypes. Another point of clinical importance is the allele frequency of this variant in the population; in a survey of 5435 unrelated Swiss white subjects,[54] the carriage frequency of *rs8099917* was 17%. According to the Human Haplotype Map project (HapMap; www.hapmap.org), 15% to 19% of white people carry the rs8099917 minor allele. Carriage frequencies differ considerably (range, 2%–31%) in different ethnicities. These differences might therefore contribute to divergent clearance rates observed across populations.[5,6,15]

Taken together, the increasing evidence for the role of IFN-λ for both spontaneous and treatment-induced control of HCV infection opens new avenues for prognosis and treatment of HCV infection.

Supplementary Material

Note: To access the supplementary material accompanying this article, visit the online version of *Gastroenterology* at www.gastrojournal.org, and at doi: 10.1053/j.gastro.2009.12.056.

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Conflicts of interest

The authors disclose no conflicts.

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Appendix 1. Cohort Members

The members of the Swiss HCV Cohort Study are F. Negro (Chairman, University Hospitals, Geneva, Switzerland), A. Hadengue, L. Kaiser, L. Rubbia–Brandt, D. Moradpour (Lausanne, Chairman of Scientific Committee), P. Burgisser, P. Francioli, S. Estoppey-Younes, F. Schoni–Affolter, M. Rickenbach (Head of Data Center), G. Martinetti, A. Cerny, V. Masserey Spicher, M. Gorgievski, J. F. Dufour, H. Hirsch, H. Heim, B. Helbling, B. Müllhaupt, S. Regenass, R. Malinverni, C. Meyenberger, J. Borovicka, G. Dollenmaier, and G. Cathomas.

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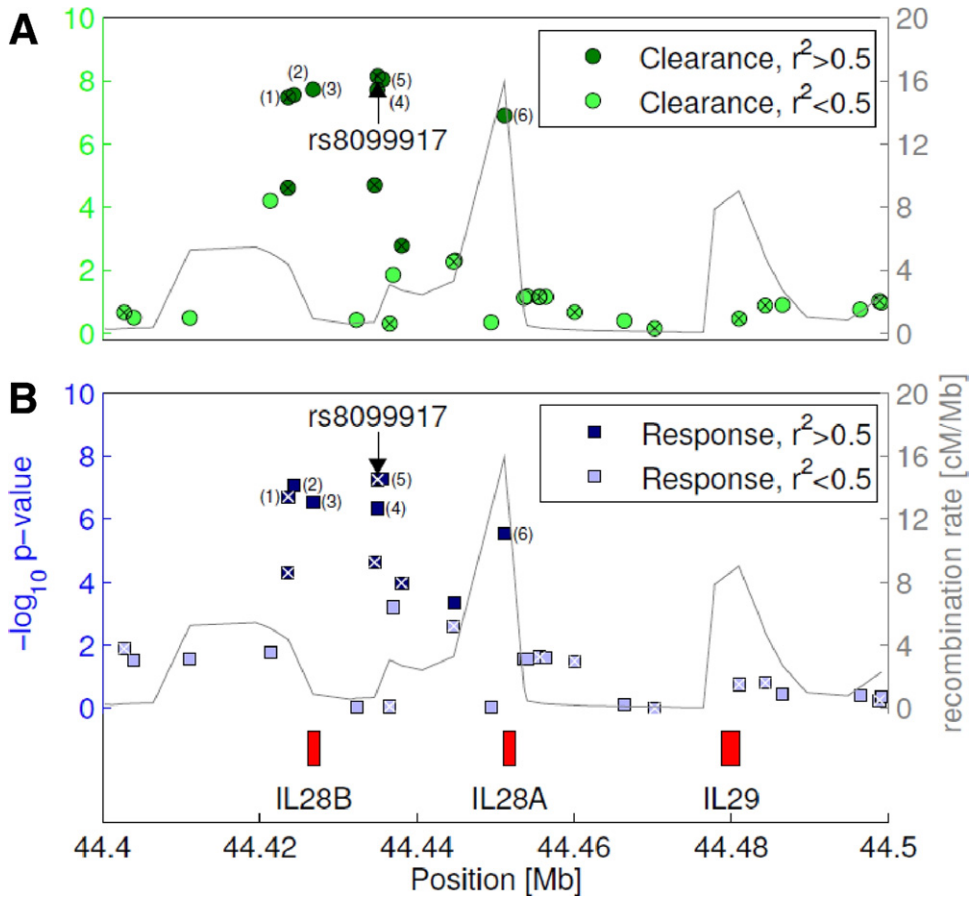
Supplementary Table 1. Primers Used to Amplify and Resequence *IL28B*

Region amplified	Polymerase chain reaction primers	Size (*base pairs*)	Temperature (*°C*)
Promoter *IL28B*	Forward, 5′-GGTGGCCTGAGTTTCAGTTC-3′; reverse, 5′-CCCGGTCATGTCTGTGTC-3′	1500	62
Exons and introns *IL28B*	Forward, 5′-GTGGGCAGCCTCTGCATTC-3′; reverse, 5′-CAAATACATAAATAGCGACTGGGTGAC-3′	1476	62
3′UTR *IL28B*	Forward, 5′-CTTCCGCCAGTCATGCAAC-3′; reverse, 5′- AGCAGGCACCTTGAAATGTC-3′	1450	65
Region sequenced	**Sequencing primers**	**Size (*base pairs*)**	**Temperature (*°C*)**
Promoter part 1	Forward, 5′-GGTGGCCTGAGTTTCAGTTC-3′; reverse, 5′-TGCCCAGAGGCCAATATTTC-3′	512	50
Promoter part 2	Forward, 5′-CCTTCGTCACACCTCAATTC-3′; reverse, 5′-GGAAGGTATGTTCCCAAGAG-3′	581	50
Promoter part 3	Forward, 5′-GAGCAGGTGGAATCCTCTTG-3′; reverse, 5′-CCCGGTCATGTCTGTGTC-3′	529	50
Exons-introns part 1	Forward, 5′-GTGGGCAGCCTCTGCATTC-3′; reverse, 5′-AGCAGAAGCGACTCTTCC-3′	540	50
Exons-introns part 2	Forward, 5′-GGCTAACCTGTGCCTTTG-3′; reverse, 5′-GGAGCTGGGAGAGGATATG-3′	505	50
Exons-introns part 3	Forward, 5′-CTGACGCTGAAGGTTCTG–3′; reverse, 5′-CAAATACATAAATAGCGACTGGGTGAC-3′	577	50
3′UTR part 1	Forward, 5′-CTTCCGCCAGTCATGCAAC-3′; reverse, 5′-TCAAGTGATCCTCCCAACTC-3′	582	50
3′UTR part 2	Forward, 5′-CCTGGATGTGATTGCTCAAG-3′; reverse, 5′-GGTGGAGAATGACACTCTG-3′	565	50
3′UTR part 3	Forward, 5′-TGAGCTGCTGGAACAAAG-3′; reverse, 5′-AGCAGGCACCTTGAAATGTC-3′	443	50

UTR, untranslated region.



Supplementary Figure 1. (*A*) Association of SNPs with chronic (vs spontaneously resolved) HCV infection in the *IL28B/A* and *IL29* locus. The lowest *P* values are located within a low recombination rate region that encompasses the *IL28B* gene. The area is separated from the *IL28A* gene by a high recombination peak. This suggests that the association targets a haplotype block containing *IL28B*. Thus, *IL28B* is more likely to be associated with spontaneous clearance than *IL28A*. Measured SNPs, as opposed to inferred SNPs, are indicated by an X. (*B*) The association with treatment failure shows the same pattern as for chronic HCV infection and further supports an association with *IL28B*. Numbers in brackets are (1) *rs12980275,* (2) *rs8105790,* (3) *rs11881222,* (4) *rs8113007,* (5) *rs7248668,* and (6) *rs576832*.

Supplementary Table 2. Demographics

	End point spontaneous hepatitis C clearance						End point response to therapy	
	Mono-infected		Coinfected		Total		Mono-infected	
	Chronic infection	Spontaneous clearance	Chronic infection	Spontaneous clearance	Chronic infection	Spontaneous clearance	Nonresponders	Sustained responders
n	779	135	236	212	1015	347	168	297
Median age (*y*) (interquartile range)	44.66 (14.17)	43.61 (16.81)	33.00 (10.00)	34.00 (7.00)	41.86 (14.71)	36.00 (12.00)	46.80 (12.02)	42.66 (14.71)
Male sex	489 (0.63)	61 (0.45)	158 (0.67)	110 (0.52)	647 (0.64)	171 (0.49)	114 (0.68)	181 (0.61)
Hepatitis B surface antigen positive[a]	8 (0.02)	3 (0.03)	8 (0.04)	20 (0.10)	16 (0.03)	23 (0.08)	2 (0.02)	3 (0.02)
HCV genotypes								
1	373 (0.48)	NA	98 (0.42)	NA	471 (0.46)	NA	101 (0.60)	87 (0.29)
2	80 (0.10)		7 (0.03)		87 (0.09)		8 (0.05)	52 (0.18)
3	223 (0.29)		71 (0.30)		294 (0.29)		28 (0.17)	134 (0.45)
4	67 (0.09)		24 (0.10)		91 (0.09)		18 (0.11)	16 (0.05)
Other/unknown	36 (0.05)		36 (0.15)		72 (0.07)		13 (0.08)	8 (0.03)
Log HCV RNA (median, IQR)[b]	5.87 (0.98)	0 (0)	6.09 (1.16)	0 (0)	5.90 (1.03)	0 (0)	5.94 (0.85)	5.83 (1.15)
Heavy drinker[c]							30 (0.21)	32 (0.12)
Liver biopsy[d]							122 (0.73)	202 (0.68)
Severe fibrosis							54 (0.44)	54 (0.27)
Severe inflammation							22 (0.18)	45 (0.22)
Steatosis							2 (0.50)	20 (0.40)

NOTE. All values are expressed as n (proportion).
NA, not applicable.
[a]Information on hepatitis B surface antigen before the measurement of HCV RNA was missing in 466 individuals.
[b]HCV RNA at set point (for clearance end point) and before treatment (for treatment end point).
[c]Heavy drinker was defined as use of more than 40 g alcohol per day for more than 5 years.
[d]Biopsy data before treatment were missing in 128 patients. Severe fibrosis and inflammation were defined by a METAVIR score $\geq$3 and steatosis by the presence of steatosis in >5% of hepatocytes.

Supplementary Table 3. Top 20 Hits ($P < 5 \times 10^{-5}$) by Chromosome for the Association With Chronic Versus Spontaneously Resolved HCV Infection

rs no.	Chromosome	Position (*base pairs*)	Gene	Distance (*kilobase*)	Minor allele frequency	r2-hat	Alleles		*P* value	Effect size	SE
rs3806575	2	217071179	*RPL37A*	1	0.05	0.67	G	C	4.29E-05	−1.00	0.24
rs3765098	3	1390195	*CNTN6*	0	0.48	1.00	T	C	4.51E-05	−0.41	0.10
rs4685148	3	14403792	*SLC6A6*	15	0.09	0.95	T	C	3.11E-05	−0.77	0.19
rs661777	3	52440226	*SEMA3G*	2	0.06	0.86	G	A	2.84E-05	1.14	0.27
rs7621695	3	196961660	*MUC4*	0	0.36	0.34	G	A	2.52E-05	−0.77	0.18
rs4689126	4	7518216	*SORCS2*	0	0.27	0.90	T	C	3.20E-05	−0.52	0.12
rs9349739	6	9705195	*OFCC1*	300	0.49	0.99	T	C	5.01E-05	0.42	0.10
rs880122	6	165988331	*PDE10A*	0	0.03	0.48	C	A	4.25E-05	−1.49	0.36
rs4278174	8	5910879	*MCPH1*	341	0.15	0.92	T	A	2.68E-05	−0.58	0.14
rs6984231	8	10264638	*MSRA*	0	0.46	0.93	G	A	1.72E-05	0.43	0.10
rs6584313	10	83542130	*NRG3*	83	0.22	0.97	G	A	4.78E-05	0.51	0.12
rs4463881	12	90832436	*BTG1*	229	0.29	0.95	T	C	3.61E-05	0.45	0.11
rs11612078	12	112692736	*RBM19*	52	0.13	0.90	G	A	6.19E-06	−0.68	0.15
rs9538873	13	60165634	*TDRD3*	120	0.19	0.94	T	G	3.49E-05	0.62	0.15
rs765771	16	63647863	*CDH11*	0	0.40	0.96	T	C	1.47E-05	0.47	0.11
rs8091335	18	31282121	*C18orf37*	20	0.49	0.97	C	A	2.85E-05	0.42	0.10
rs8099917	**19**	**44435005**	***IL28B***	**8**	**0.21**	**1.00**	**T**	**G**	**6.07E-09**	**0.84**	**0.14**
rs1297152	21	14615347	*STCH*	50	0.41	1.00	T	C	4.20E-05	−0.42	0.10
rs7280694	21	42418487	*UMODL1*	0	0.35	0.98	T	C	1.47E-05	0.46	0.11
rs5753723	22	30332294	*SFI1*	0	0.20	1.00	T	C	4.58E-05	0.48	0.12

NOTE. Only one SNP is reported for each locus. The top hit *rs8099917* appears in bold.
r2-hat, imputation accuracy.

Supplementary Table 4. Association of SNPs in the *IL28/IL29* Locus With Chronic Versus Spontaneously Resolved HCV Infection

Rs number	Chromosome	Position [bp]	Imputed	Gene	Distance [kb]	MAF	r2-hat	Alleles		P-value	Effect size	SE
rs11879005	19	44402743	0	SYCN	16	0.42	1	T	C	2.49E-01	−0.12	0.1
rs12975799	19	44403977	1	SYCN	17	0.42	0.92	G	A	3.29E-01	0.1	0.11
rs11083519	19	44411103	1	IL28B	15	0.42	0.88	T	A	3.39E-01	−0.1	0.11
rs955155	19	44421319	1	IL28B	5	0.25	0.92	G	A	7.39E-05	0.49	0.12
rs12972991	19	44423587	0	IL28B	3	0.25	1	C	A	7.23E-05	−0.47	0.12
rs12980275	19	44423623	0	IL28B	2	0.32	1	G	A	3.03E-08	−0.63	0.11
rs8105790	19	44424341	1	IL28B	2	0.24	0.81	T	C	5.24E-08	0.81	0.15
rs11881222	19	44426763	1	IL28B	0	0.32	0.98	G	A	2.48E-08	−0.64	0.12
rs10853727	19	44432303	1	IL28B	5	0.10	0.93	T	C	3.42E-01	0.16	0.17
rs8109886	19	44434602	0	IL28B	7	0.45	1	C	A	1.51E-05	0.44	0.1
rs8113007	19	44434943	1	IL28B	7	0.31	0.98	T	A	1.82E-08	−0.66	0.12
rs8099917	**19**	**44435005**	**0**	**IL28B**	**8**	**0.21**	**1**	**T**	**G**	**6.07E-09**	**0.84**	**0.14**
rs7248668	19	44435661	1	IL28B	8	0.21	0.97	G	A	7.59E-09	0.84	0.15
rs16973285	19	44436536	0	IL28B	9	0.10	1	T	C	5.22E-01	−0.11	0.16
rs10853728	19	44436986	1	IL28B	10	0.30	0.41	G	C	8.22E-03	−0.44	0.16
rs4803223	19	44438059	0	IL28B	11	0.16	0.98	G	A	1.68E-03	−0.46	0.15
rs12980602	19	44444660	0	IL28A	6	0.22	1	T	C	6.24E-03	0.34	0.12
rs4803224	19	44444854	1	IL28A	6	0.27	0.75	G	C	5.12E-03	0.36	0.13
rs664893	19	44449412	1	IL28A	2	0.10	0.79	G	A	4.80E-01	−0.13	0.19
rs576832	19	44451122	1	IL28A	0	0.32	0.7	G	C	9.04E-08	0.71	0.13
rs11671087	19	44453630	1	IL28A	1	0.21	0.99	T	C	6.04E-02	0.23	0.12
rs251910	19	44453989	1	IL28A	1	0.27	0.94	T	C	4.69E-02	−0.23	0.12
rs7359953	19	44455553	1	IL28A	3	0.21	1	G	A	5.87E-02	0.23	0.12
rs7359950	19	44455605	0	IL28A	3	0.21	1	T	C	4.82E-02	−0.24	0.12
rs2099331	19	44456390	1	IL28A	4	0.21	1	T	G	5.50E-02	0.24	0.12
rs11665818	19	44460056	0	IL28A	7	0.19	1	G	A	1.78E-01	0.17	0.13
rs570880	19	44466370	1	IL29	12	0.10	0.86	T	A	2.80E-01	−0.19	0.18
rs503355	19	44470224	0	IL29	9	0.07	1	T	C	6.58E-01	−0.09	0.21
rs30461	19	44480955	0	IL29	0	0.11	1	G	A	2.27E-01	−0.19	0.16
rs194014	19	44484300	0	IL29	3	0.09	1	G	A	8.03E-02	0.3	0.17
rs251903	19	44486490	1	LRFN1	3	0.09	0.95	G	C	7.51E-02	0.3	0.17
rs12979175	19	44496394	1	LRFN1	0	0.07	0.96	G	A	1.17E-01	0.3	0.19
rs39587	19	44498837	0	LRFN1	1	0.09	0.99	G	A	4.90E-02	−0.34	0.17
rs30480	19	44499053	1	LRFN1	1	0.09	0.96	G	A	5.73E-02	0.32	0.17

NOTE. The list contains both imputed and measured SNPs. The top hit *rs8099917* appears in bold.

Supplementary Table 5. Association of the *rs8099917* SNP With Spontaneous and Treatment-Induced Control of HCV Infection and Association of *rs8099917* With Chronic Versus Spontaneously Resolved HCV Infection

	Chronic infection, n (proportion)		Spontaneous clearance, n (proportion)		Inheritance	Multivariate[a] OR (95% CI)	*P* value
Mono-infected							
IL28B-7554 genotype							
TT	441	0.57	103	0.76	Recessive	3.69 (0.86–15.79)	7.79E-02
GT	297	0.38	30	0.22	Dominant	2.75 (1.75–4.32)	1.07E-05
GG	41	0.05	2	0.01	Additive[b]	2.49 (1.64–3.79)	1.96E-05
Coinfected							
IL28B-7554 genotype							
TT	145	0.61	166	0.78	Recessive	6.36 (1.40–28.84)	1.65E-02
GT	78	0.33	44	0.21	Dominant	2.22 (1.45–3.48)	2.35E-04
GG	13	0.06	2	0.01	Additive[b]	2.16 (1.47–3.18)	8.25E-05
Combined[c]							
IL28B-7554 genotype							
TT	586	0.58	269	0.78	Recessive	4.79 (1.68–13.67)	3.37E-03
GT	375	0.37	74	0.21	Dominant	2.46 (1.80–3.35)	1.23E-08
GG	54	0.05	4	0.01	Additive[b]	2.31 (1.74–3.06)	6.07E-09

Association of *rs8099917* with treatment failure

	Treatment failure, n (proportion)		Treatment success, n (proportion)		Percentage of failure	Inheritance	Multivariate[d] OR (95% CI)	*P* value
Mono-infected								
IL28B-7554 genotype								
TT	71	0.42	201	0.68	26	Rec	1.80 (0.51–6.37)	3.62E-01
GT	85	0.51	86	0.29	50	Dom.[b]	5.19 (2.90–9.30)	3.11E-08
GG	12	0.07	10	0.03	55	Add	3.45 (2.12–5.61)	6.30E-07

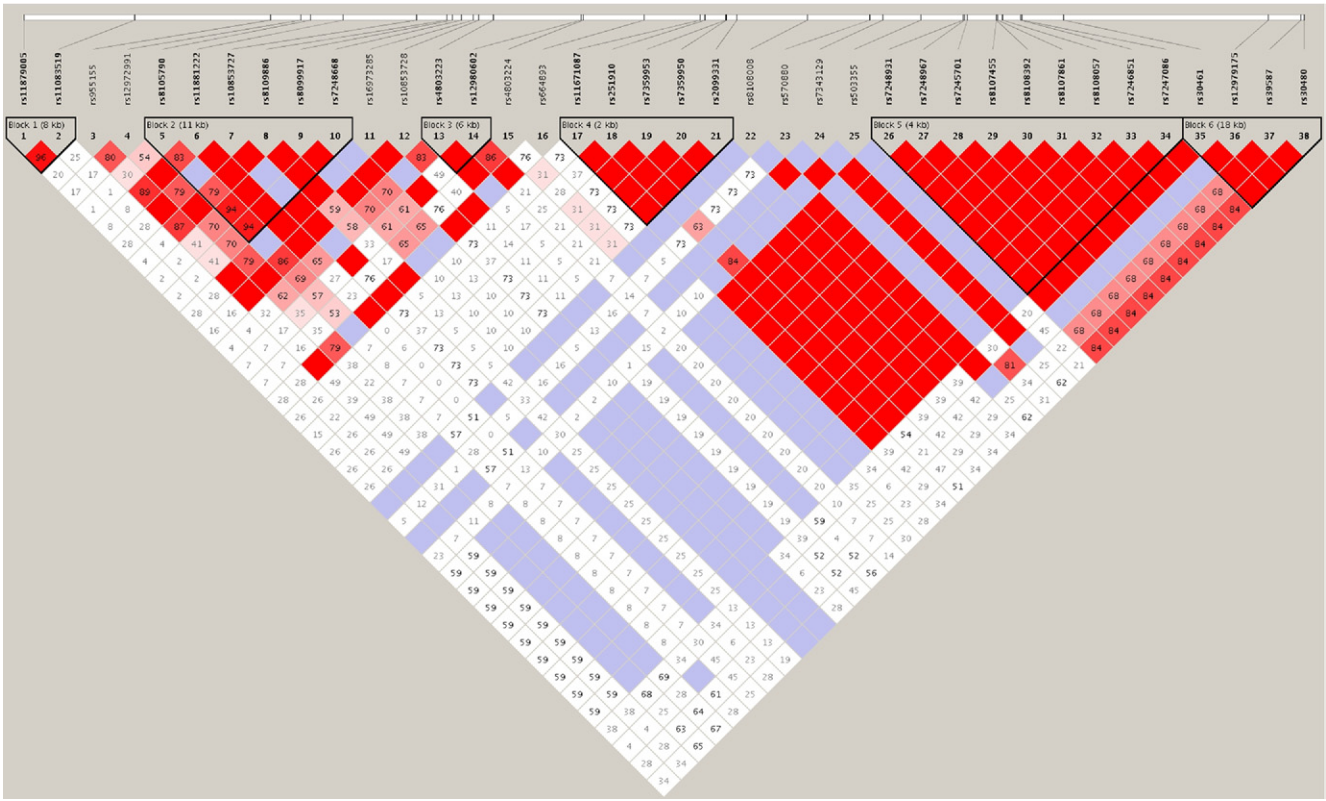
NOTE. Because patients with clearance are frequently undetected in the population, percentages of chronic infection versus spontaneous clearance were not calculated. For the end point of spontaneous HCV clearance, we could not adjust for age because the time point of acute HCV infection is unknown in most cases.

[a]Logistic regression estimate adjusted for population stratification and sex.

[b]Most likely model.

[c]Meta-analysis with inverse variance weighting.

[d]Logistic regression estimate adjusted for population stratification, sex, age, viral genotype, HCV RNA level, and liver fibrosis stage.



Supplementary Figure 2. Pairwise LD (r^2) pattern of the *IL28* region is shown.