

ORIGINAL ARTICLE

IL28B genetic variations are associated with high sustained virological response (SVR) of interferon- α plus ribavirin therapy in Taiwanese chronic HCV infectionJ-Y Chen¹, C-Y Lin², C-M Wang³, Y-T Lin¹, S-N Kuo¹, C-F Shiu⁴, S-W Chang⁴, J Wu⁵ and I-S Sheen²

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Chronic hepatitis C virus (HCV) infection patients exhibit different sustained virological responses (SVRs) following the treatment with pegylated interferon- α (IFN- α) and ribavirin. Genome-wide association studies consistently linked SVR of IFN- α -based therapy to the IL28B single-nucleotide polymorphisms (SNPs) on chromosome 19q.13 in various populations. This study was undertaken to investigate the association of IL28B SNPs with SVR in a cohort of Taiwanese chronic HCV patients. Ten SNPs of IL28B were genotyped in 728 chronic HCV patients and 960 healthy controls. Genotype distributions, allele frequencies and haplotypes were tested for SVR and susceptibility in Taiwanese chronic HCV patients. Non-genotype 1 infection (adjusted $P = 3.3 \times 10^{-12}$, odds ratio (OR) 0.179; 95% confidence interval (CI): 0.110–0.290) and low HCV viral load ($< 400\,000\text{ IU ml}^{-1}$) (adjusted $P = 3.5 \times 10^{-9}$, OR 0.299; 95% CI: 0.200–0.446) were two major factors identified for high SVR. Notably, eight IL28B SNPs including previously described disease-associated SNPs (Trend test $P = 0.005$) were significantly associated with SVR. Our data indicate that IL28B polymorphisms are the essential contributing factors for high SVR in Taiwanese chronic HCV patients. Combination of virus genotyping and host genetic data may be used to select the optimal treatment regimes in IFN-based therapy.

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Introduction

Hepatitis C virus (HCV) infection, the leading cause of chronic liver disease, is emerging as a global concern of public health inflicting up to 3% human populations.¹ HCV infection usually appears as an asymptomatic disease, which accompanies the development of chronic persistent viremia in majority individuals despite substantial virus and host cellular immune responses.^{2–4} HCV chronic infection associates with an intra-hepatic inflammatory infiltration, which is frequently followed by progressive fibrosis that leads to liver cirrhosis and ultimately to the development of hepatocellular carcinoma.^{5–7}

HCV infection induces the production of interferons (IFNs), especially IFN α (IFN- α), in part through viral nucleic acid interactions with distinct pathogen-associated molecular pattern recognition receptors, such as Toll-like receptors on cell surface and/or intracellular sensors, such as retinoic acid-inducible gene I like helicases in the infected host cells.^{8–11} The IFN signaling pathways activate IFN-stimulated genes (ISGs), which play critical roles in the host innate defense against HCV infection through repressing viral replication and enhancing cellular immune responses.^{9,11,12} Pegylated IFN- α plus ribavirin therapy became the standard therapy for HCV viral infection recently. However, the therapy failed to cure all patients who underwent the treatment. In addition, patients may encounter specific adverse effects that require therapy withdrawal.¹³ The interplay of the virus, environmental factors and the host immunity is implicated as the key process for the natural heterogeneity of viral clearance and liver injury.^{14–19} It remains a critical issue with regard to the viral persistence and the response to anti-viral therapy in individual patient.

Interferon λ (IFN- λ) is classified as the type 3 IFN that includes three subtypes (IFN- λ 1, 2 and 3). IFN- λ s have pleiotropic cellular functions. The ligand receptor

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interaction triggers signaling pathways similar to those by IFN- α and β .^{20,21} IFN- λ s are produced and expressed in antigen-presenting cells. IFN- λ s have lower antiviral activities than IFN- α . However, IFN- λ s have critical roles in fighting against viral infection.^{22–24} Recently, four genome-wide association studies concurrently provided the overwhelming evidence that single-nucleotide polymorphisms (SNPs) of IFN- λ 3 (also known as IL28B) on chromosome 19q13 contribute to IFN treatment response and spontaneous HCV clearance in HCV infection.^{25–29} Meanwhile, IL28B genetic variations influence the IL28B mRNA expressions, which may have a role in the regulation of intra-hepatic ISG expression.^{26,27} IL28B SNPs seem to impact the treatment response to pegylated IFN- α /ribavirin for chronic HCV infection among several ethnic populations.^{25–29} In this study, we investigated whether the IL28B polymorphisms are associated with susceptibility to HCV infection and with response to the therapy of IFN and ribavirin in Taiwanese chronic HCV infection patients.

Results

Clinical characteristics of chronic HCV infection patients

This study recruited 728 Taiwanese patients with chronic HCV infection including 444 men and 284 women who received IFN- α plus ribavirin therapy. The mean ages on therapy were 51.2 ± 10.4 (ranged 19.2–76.5) years old. Among them, 126 patients (112 patients were treated for a period of 24 weeks and 14 patients for 48 weeks) received treatment of INTRON 5MU three times a week in combination of 1200 mg ribavirin daily. The remaining 602 patients received ribavirin (1200 mg) daily and pegylated IFN- α every week (499 patients were treated for a period of 24 weeks and 103 patients for 48 weeks). Of the total 728 chronic HCV patients, 559 (76.8%) revealed sustained virological response (SVR) to IFN (INTRON or pegylated IFN- α) plus ribavirin therapy. In addition, 424 (58.2%) were identified as HCV genotype 1 viral infection and 318 (43.7%) were detected with viral load over 400 000 IU ml⁻¹ (Table 1). The liver biopsies showed that 246 (33.8%) were in cirrhosis stage, 114

(15.7%) in severe fibrosis stage and 368 (50.5%) in mild fibrosis stage.

Clinical characteristics affect SVR during the treatment of chronic HCV infection

We examined the possible factors that may contribute to the SVR of chronic HCV infection. As shown in Table 2, age at therapy and gender were important host factors that affected the SVR. Patients with younger age at therapy were more likely to have higher SVR rate in chronic HCV infection (adjusted $P = 0.0004$, odds ratio (OR) 0.962; 95% confidence interval (CI): 0.942–0.983). The SVR rate tended to be higher (81.3%) in men than in women (69.8%) (adjusted $P = 0.0093$, OR 1.705; 95% CI: 1.140–2.549). Non-cirrhosis (mild and severe fibrosis) is another critical factor that demonstrated high SVR in comparison with cirrhosis (adjusted $P = 4.1 \times 10^{-6}$, OR 0.379; 95% CI: 0.251–0.573). Body mass index below 27 kg m⁻² showed modest effect on the higher SVR (adjusted $P = 0.0486$, OR 0.616; 95% CI: 0.381–0.997). There was no significant SVR difference between the patients with INTRON plus ribavirin (76.6%) and those with pegylated IFN- α plus ribavirin (77.8%) albeit the percentage of patients with high viral RNA were lower in INTRON plus ribavirin group (19.0%) than that in pegylated IFN- α plus ribavirin (48.8%) group. Viral genotypes were significantly associated with SVR. Patients with non-HCV genotype 1 infection (91.4%; 278 of 304) were more likely to reveal SVR than the patients with HCV genotype 1 infection (66.3%) (adjusted $P = 3.6 \times 10^{-12}$, OR 0.178; 95% CI: 0.110–0.290). In addition, 86.6% of the patients with viral load <400 000 IU ml⁻¹ demonstrated SVR as compared with 64.2% of the patients with HCV viral load over 400 000 IU ml⁻¹ (adjusted $P = 2.3 \times 10^{-9}$, OR 0.292; 95% CI: 0.195–0.437). Therefore, viral load appeared to contribute significantly to the SVR in the therapy.

IL28B polymorphisms are associated with chronic HCV infection

Ten SNPs of IL28B genetic variations were genotyped in 728 chronic HCV infection patients and 960 healthy

Table 1 Clinical characteristics and SVR analysis of Taiwanese patients with chronic HCV infection

Clinical characteristic	Total (N = 728)	SVR positive (N = 559)	SVR negative (N = 169)	P-value
Age on IFN therapy	51.2 ± 10.4	50.1 ± 10.5	54.8 ± 9.3	<0.001*
Male	444 (61.0%)	361 (64.6%)	83 (49.1%)	<0.001*
Liver cirrhosis	246 (33.8%)	155 (27.7%)	91 (53.8%)	<0.001*
HCV genotype:				
Genotype 1	424 (58.2%)	281 (50.3%)	143 (84.6%)	<0.001*
Non-genotype 1	304 (41.8%)	278 (49.7%)	26 (15.4%)	
HCV RNA ≥ 0.4 miu	318 (43.7%)	204 (36.5%)	114 (67.5%)	<0.001*
BMI ≥ 27 kg m ⁻²	146 (20.1%)	106 (19.0%)	40 (23.7%)	0.181
Adherence	483 (66.3%)	386 (69.1%)	39 (23.1%)	0.005*
Regimen				
INTRON+RBV	126 (17.3%)	98 (17.5%)	28 (16.6%)	0.772
PEG-IFN+RBV	602 (82.7%)	461 (82.5%)	141 (83.4%)	

Abbreviations: BMI, body mass index; HCV, hepatitis C virus; PEG IFN, pegylated interferon; RBV, ribavirin; SVR, sustained virological response.

* $P < 0.05$.

Table 2 Clinical characteristics significantly affect SVR by multiple varieties analysis in 728 Taiwanese chronic HCV infection patients

Clinical characteristics	N	Stepwise logistic regression		
		OR	95% CI	P-value
Age on IFN therapy	728	0.962	0.942–0.983	0.0004
Gender: female	284	1.705	1.140–2.549	0.0093
BMI: below 27 kg m ⁻²	582	0.616	0.381–0.997	0.0486
HCV RNA viral load ≤ 400 000 IU ml ⁻¹	410	0.292	0.195–0.437	2.3 × 10 ⁻⁹
HCV genotype: Non-genotype 1	304	0.178	0.110–0.290	3.6 × 10 ⁻¹²
Liver biopsy: non-cirrhosis	482	0.379	0.251–0.573	4.1 × 10 ⁻⁶

Abbreviations: BMI, body mass index; CI, confidence interval; HCV, hepatitis C virus; OR, odds ratio; SVR, sustained virological response.

Table 3 Allele and genotype distributions of IL28B SNPs in 728 Taiwanese chronic HCV infection patients and 960 normal controls

SNP marker	Risk allele	RAF (normal)	RAF (HCV)	P _{trend}	Genotypes/alleles	Age, sex adjusted	
						P-value	OR (95% CI)
rs28416813	C	0.975	0.999	1.0 × 10 ⁻⁶	C/G+G/G vs C/C	1.5 × 10 ⁻⁵	0.012 (0.002–0.089)
					G vs C	6.4 × 10 ⁻⁷	0.026 (0.006–0.110)
rs8099917	T	0.936	0.947	0.2006	G/G vs G/T+T/T	0.0010	0.034 (0.004–0.254)
					G vs T	0.0425	0.713 (0.515–0.989)

Abbreviations: CI, confidence interval; HCV, hepatitis C virus; OR, odds ratio; RAF (normal) and RAF (HCV), risk allele frequency in normal controls and HCV patients; SNP, single-nucleotide polymorphism.

P_{trend} value were calculated by the Cochran–Armitage trend test with permutation = 100 000.

controls. As shown in Table 3, genotype distributions and allele frequencies of two IL28B SNPs were significantly different between patients with chronic HCV infection and the normal healthy donors. The C allele of SNP rs28416813 appeared to be a risk allele for HCV infection (Trend test $P = 1.0 \times 10^{-6}$ with 100 000 permutations; G vs C adjusted $P = 6.4 \times 10^{-7}$, OR 0.026, 95% CI: 0.006–0.110; genotypes CG+GG vs CC adjusted $P = 1.5 \times 10^{-5}$, OR 0.012, 95% CI: 0.002–0.089). In addition, the SNP rs8099917 genotypes appear to be a predisposition factor for HCV infection (genotype GG vs GT + TT adjusted $P = 0.001$, OR 0.034; 95% CI: 0.004–0.254). Therefore, the SNP rs8099917 GG could be defined as the protective genotype. Notably, the SNP rs8099917 GG genotype frequency of the normal control males tested negative for HCV antibody was 21.5 times of that in the male patients with HCV infection (Table 4). Significant deviations from Hardy–Weinberg equilibrium in genotype and allele distributions were observed for SNP rs28416813 and rs8099917 in both HCV patients and normal controls ($P < 0.05$).

IL28B polymorphisms are associated with SVR in the treatment of chronic HCV infection

Patients with chronic HCV infection may manifest heterogeneous clinical characteristics and exhibit different SVRs to IFN therapy. Hence, we stratified patients into two groups in accordance with SVR to the treatment. As shown in Table 3, genotype distributions of the eight SNPs (rs12980275, rs8105790, rs11881222, rs4803219, rs12979860, rs8099917, rs7248668 and rs10853728) were significantly different between SVR positive and negative patients (Trend test $P = 0.005$ with 100 000 permutations). The advantageous alleles of IL28 polymorphisms had similarly high frequencies in SVR positive groups

(Table 5). Consequently, we examined whether IL28B SNP haplotypes were associated with SVR based on linkage disequilibrium blocks (Figure 1). As shown in Table 6, IL28B haplotypes ACC (rs11881222, rs4803219 and rs12979860), AT (rs12980275 and rs8105790), ATA (rs12980275, rs8105790 and rs11881222) and TA (rs8105790 and rs11881222) revealed as low responder alleles. Our data suggest that genetics of IL28B may impact the SVR in the treatment of HCV infection in Taiwanese.

IL28B polymorphisms are associated with HCV genotypes of chronic HCV infection

We also investigated possible interactions of IL28B polymorphisms with other clinical characteristics of chronic HCV infection in Taiwanese. We observed significant association ($P < 0.05$) between HCV genotypes and IL28B SNP rs8099917 (Trend test $P = 0.03$ with 100 000 permutations; G vs T adjusted $P = 0.0318$, OR 1.729, 95% CI: 1.049–2.851) and between HCV genotypes and rs7248668 (Trend test $P = 0.028$ with 100 000 permutations; A vs G adjusted $P = 0.0267$, OR 1.777, 95% CI: 1.069–2.956). Our data suggest that IL28B gene products may associate with certain strains of HCV infection. Next, we performed multivariate analysis to comparatively evaluate the independent contribution of the significant IL-28B SNPs and the non-genetic clinical characteristics. Notably, rs12979860 is the only IL28B SNP showed significant association in multivariate analysis ($P = 2.3 \times 10^{-8}$, OR 0.177, 95% CI: 0.096–0.324).

Discussion

Chronic HCV infection involves in a complex interaction of virus with host innate and adaptive immunity.

Table 4 Genotype frequencies of IL28B SNPs in normal controls and patients with chronic HCV infection, SVR positive, SVR negative

Clinical characteristic/ IL28B SNPs	SVR positive			SVR negative			HCV			Normal		
	Male (N = 360)	Female (N = 199)	Total (N = 559)	Male (N = 83)	Female (N = 86)	Total (N = 169)	Male (N = 443)	Female (N = 285)	Total (N = 728)	Male (N) ^a	Female	Total
	<i>rs12980275</i>	0.903	0.950	0.920	0.807	0.791	0.799	0.885	0.902	0.892	0.870	0.883
AA	0.097	0.045	0.079	0.193	0.209	0.201	0.115	0.095	0.107	0.127	0.112	0.120
AG	0.000	0.005	0.002	0.000	0.000	0.000	0.000	0.004	0.001	0.004	0.005	0.004
GG												
<i>rs8105790</i>	0.000	0.000	0.000	0.012	0.000	0.006	0.002	0.000	0.001	0.004	0.002	0.003
CC	0.094	0.045	0.077	0.205	0.186	0.195	0.115	0.088	0.104	0.101	0.105	0.103
CT	0.906	0.955	0.923	0.783	0.814	0.799	0.883	0.912	0.894	0.895	0.892	0.894
TT												
<i>rs11881222</i>	0.911	0.960	0.928	0.795	0.791	0.793	0.889	0.909	0.897	0.881	0.892	0.886
AA	0.089	0.040	0.072	0.193	0.209	0.201	0.108	0.091	0.102	0.115	0.106	0.111
AG	0.000	0.000	0.000	0.012	0.000	0.006	0.002	0.000	0.001	0.004	0.002	0.003
GG												
<i>rs8103142</i>	0.000	0.000	0.000	0.012	0.000	0.006	0.002	0.000	0.001	0.006	0.002	0.004
CC	1.000	1.000	1.000	0.988	1.000	0.994	0.998	1.000	0.999	0.994	0.998	0.996
CT	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
TT												
<i>rs28416813</i>	1.000	1.000	1.000	0.998	1.000	0.994	0.998	1.000	0.999	0.914	0.998	0.953
CC	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.082	0.000	0.044
CG	0.000	0.000	0.000	0.012	0.000	0.006	0.002	0.000	0.001	0.004	0.002	0.003
GG												
<i>rs4803219</i>	0.917	0.960	0.932	0.807	0.791	0.799	0.896	0.909	0.901	0.88	0.881	0.883
CC	0.083	0.040	0.068	0.193	0.209	0.201	0.104	0.091	0.099	0.115	0.119	0.117
CT	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
TT												
<i>rs12979860</i>	0.917	0.960	0.932	0.795	0.779	0.787	0.894	0.905	0.898	0.885	0.892	0.888
CC	0.083	0.040	0.068	0.193	0.221	0.207	0.104	0.095	0.100	0.109	0.105	0.107
CT	0.000	0.000	0.000	0.012	0.000	0.006	0.002	0.000	0.001	0.006	0.002	0.004
TT												
<i>rs8099917</i>	0.000	0.000	0.000	0.012	0.000	0.006	0.002	0.000	0.001	0.043	0.002	0.024
GG	0.092	0.045	0.075	0.217	0.174	0.195	0.115	0.084	0.103	0.060	0.103	0.080
GT	0.908	0.955	0.925	0.771	0.826	0.799	0.883	0.916	0.896	0.897	0.895	0.896
TT												
<i>rs7248668</i>	0.000	0.000	0.000	0.012	0.000	0.006	0.002	0.000	0.001	0.043	0.002	0.003
AA	0.092	0.035	0.072	0.169	0.221	0.195	0.106	0.091	0.100	0.098	0.097	0.097
AG	0.908	0.965	0.928	0.819	0.779	0.799	0.892	0.909	0.898	0.899	0.901	0.900
GG												

Table 5 Association of IL28B SNPs with SVR in Taiwanese patients with chronic HCV infection

SNP marker	Risk allele	RAF (NR)	RAF (SVR)	P _{trend}	Genotypes/alleles	Age, sex adjusted		Stepwise logistic regression	
						P-value	OR (95% CI)	P-value	OR (95% CI)
rs4803219	C	0.899	0.966	2.0 × 10 ⁻⁶	C/T vs C/C	3.7 × 10 ⁻⁷	0.258 (0.153–0.435)	1.4 × 10 ⁻⁷	0.191 (0.103–0.354)
					T vs C	7.4 × 10 ⁻⁷	0.284 (0.172–0.467)	2.1 × 10 ⁻⁷	0.213 (0.119–0.382)
rs12979860	C	0.891	0.966	1.0 × 10 ⁻⁶	C/T+T/T vs C/C	4.4 × 10 ⁻⁸	0.236 (0.140–0.395)	3.1 × 10 ⁻⁸	0.181 (0.099–0.331)
					T vs C	3.5 × 10 ⁻⁸	0.252 (0.155–0.411)	2.9 × 10 ⁻⁸	0.200 (0.113–0.353)
rs11881222	A	0.894	0.964	1.0 × 10 ⁻⁶	A/G+G/G vs A/A	2.1 × 10 ⁻⁷	0.256 (0.153–0.428)	1.1 × 10 ⁻⁷	0.195 (0.107–0.356)
					G vs A	1.6 × 10 ⁻⁷	0.272 (0.167–0.443)	9.0 × 10 ⁻⁸	0.214 (0.121–0.376)
rs12980275	A	0.899	0.959	4.9 × 10 ⁻⁵	A/G+G/G vs A/A	3.4 × 10 ⁻⁶	0.302 (0.182–0.500)	2.0 × 10 ⁻⁶	0.241 (0.134–0.433)
					G vs A	1.0 × 10 ⁻⁵	0.340 (0.211–0.549)	4.3 × 10 ⁻⁶	0.275 (0.158–0.476)
rs8105790	T	0.896	0.962	9.0 × 10 ⁻⁶	C/C+C/T vs T/T	1.3 × 10 ⁻⁶	0.282 (0.169–0.471)	5.5 × 10 ⁻⁷	0.222 (0.123–0.400)
					C vs T	9.5 × 10 ⁻⁷	0.298 (0.184–0.484)	7.5 × 10 ⁻⁷	0.248 (0.143–0.431)
rs8099917	T	0.896	0.962	5.0 × 10 ⁻⁶	G/G+G/T vs T/T	9.6 × 10 ⁻⁷	0.277 (0.165–0.462)	4.3 × 10 ⁻⁷	0.217 (0.120–0.392)
					G vs T	7.1 × 10 ⁻⁷	0.293 (0.180–0.476)	6.2 × 10 ⁻⁷	0.245 (0.141–0.426)
rs7248668	G	0.896	0.964	4.0 × 10 ⁻⁶	A/A+A/G vs G/G	6.1 × 10 ⁻⁷	0.268 (0.160–0.450)	3.6 × 10 ⁻⁶	0.246 (0.136–0.445)
					A vs G	4.5 × 10 ⁻⁷	0.283 (0.173–0.462)	4.5 × 10 ⁻⁶	0.270 (0.154–0.472)
rs10853728	C	0.746	0.924	0.0015	G/G+C/G vs C/C	0.0007	0.533 (0.371–0.767)	0.0006	0.485 (0.322–0.731)
					G/G vs C/G+C/C	0.0109	0.330 (0.141–0.775)	0.0117	0.291 (0.111–0.759)
					G vs C	0.0002	0.564 (0.418–0.761)	0.0002	0.527 (0.375–0.740)

Abbreviations: CI, confidence interval; HCV, hepatitis C virus; NR, no response; OR, odds ratio; RAF, risk allele frequency; SNP, single-nucleotide polymorphism; SVR, sustained virological response.

Risk allele, the allele with higher frequency in cases compared with controls.

P_{trend} value was calculated by the Cochran–Armitage trend test with 100 000 permutations.

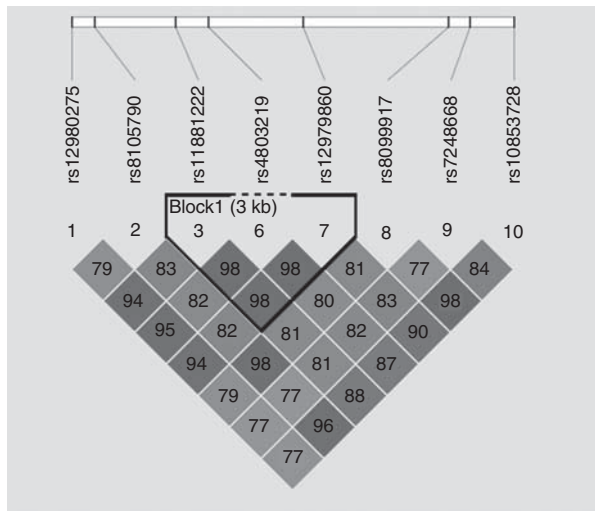


Figure 1 Pairwise linkage disequilibrium (LD) patterns for eight polymorphisms through IL28B regions.

responses collectively suggest IFN-λs are critical in suppressing viral replication during HCV chronic infection. However, the mechanisms that IL28B SNPs are associated with the response to IFN-α-based therapy remain to be elucidated in humans.

In therapy with pegylated IFN-α plus ribavirin, the adverse events and failure of response sometimes occurred.¹³ The novel HCV treatment strategies are still under development to improve sustained response rates. IFN-λs have several important roles in controlling chronic HCV infections and may be useful in future therapy. IFN-λs can enhance the subsaturating levels of IFN-α and increase the antiviral efficacy.³⁵ The combination of IFN-λs and IFN-αs may provide the additive

therapy effect through the complementary roles of two types of cytokines.^{37,38,53} As the distribution of IFN-λ receptors are restricted on plasmacytoid dendritic cells, peripheral B cell, hepatocytes and epithelial cells, IFN-λs may be used to target specific cell responses and to avoid the adverse events of therapy of IFN-αs.^{53,54}

Genome-wide association studies in independent populations confirmed that IL28B genetic variations are associated with HCV IFN-based therapy response and spontaneous clearance of HCV.^{25–29} In this study, we compared genotypes and alleles frequency of IL28B SNPs between normal controls and patients with chronic HCV infection in Taiwanese. In Caucasians and Africans, IL28B SNP rs12979860 CC genotype and C allele are associated with spontaneous resolution of HCV infection, however, no differences in genotype and allele distributions were observed for IL28B SNP rs12979860 between HCV patients and normal controls in Taiwanese. Paradoxically, rs8099917 GG genotype and rs28416813 G allele that associated with low response in Australians and Japanese were significantly enriched in Taiwanese normal male population. On the other hand, eight SNPs with strong linkage disequilibrium demonstrate significant associations with SVR on single point analysis. However, haplotype analysis failed to increase the significance of association, which is different from the results of previous studies.^{26,27} These findings suggest that various IL28B SNPs are associated with the treatment response of HCV in Taiwanese. Nevertheless, the exact causal roles of IL28B polymorphisms remain to be elucidated.

The combinational treatment of pegylated IFN-α and ribavirin is widely applied to chronic HCV infection. The treatment results in sustained clearance of virus and clinical improvement in various ethnic populations.^{13,55–58} However, the response rates showed ethnic difference with poor response in patients of African

Table 6 Association of IL28B haplotypes with SVR in patients with chronic HCV infection

Haplotype	Frequency		Associated haplotype	EM algorithm*		Logistic regression ^a		Stepwise logistic regression	
	SVR- (N = 169)	SVR+ (N = 559)		P-value	95% CI	P-value	95% CI	P-value	95% CI
rs11881222 + rs4803219 + rs12979860	0.891	0.964	ACC	<0.0001	3.31 (2.08–5.27)	6.7×10^{-8}	3.80 (2.34–6.16)	4.1×10^{-8}	4.82 (2.75–8.44)
rs12980275 + rs8105790	0.098	0.033	GTT	<0.0001	0.32 (0.20–0.51)	1.3×10^{-6}	0.29 (0.17–0.48)	3.8×10^{-7}	0.22 (0.12–0.39)
rs12980275 + rs8105790 + rs11881222	0.887	0.949	AT	0.0001	2.36 (1.53–3.62)	1.1×10^{-5}	2.73 (1.74–4.27)	5.0×10^{-6}	3.32 (1.99–5.53)
rs8105790 + rs11881222	0.885	0.949	ATA	0.0001	2.43 (1.58–3.72)	6.0×10^{-6}	2.80 (1.79–4.36)	4.0×10^{-6}	3.34 (2.00–5.56)
rs8105790 + rs11881222	0.884	0.954	TA	<0.0001	2.73 (1.76–4.22)	1.0×10^{-6}	3.15 (2.00–4.96)	4.0×10^{-7}	3.88 (2.29–6.55)

Abbreviations: CI, confidence interval; EM, expectation maximization; HCV, hepatitis C virus; OR, odds ratio; SVR, sustained virological response.

^aAdjusted for sex and age.

*P-value with 10000 permutations.

ancestry and Hispanic ancestry in comparison with patients of Caucasian ancestry.^{55,56} We observed higher SVR rates in Taiwanese chronic HCV infection who received IFN- α and ribavirin combination therapy, similar to the previous findings in Asians.^{57,58} The IL28B genetic variations affect mRNA expressions, indicating functional polymorphisms in regulatory regions may interfere with treatment responses.^{26,27} This study confirmed that IL28B SNPs are associated with SVR of HCV treatment response across all ethnic groups. The advantageous IL28B SNPs have significantly higher frequencies in Asian populations than in populations with African and Caucasian ancestry origins, which may provide explanation for the ethnic differences in SVR in IFN-based therapy among Asians, Europeans and Africans.

Recognizing the cost-effective concern and the potentially serious adverse effects, investigators and clinicians put emphasis on the importance of predicting the response to IFN-based biologic therapy. The longer duration of treatment achieved significantly higher SVR rates than the shorter course therapy, which may indicate the specific refractory cases with genotype 1 infection.^{58,59} Human genome variations explain part of the different effective responses. The combination of data including virus genotypes, viral load, immunological cellular and gene expression changes, and host genetic variations may be critical to determine individual appropriate treatment doses and duration, which is valuable to minimize the adverse effects of biologic agents. This study could provide another critical evidence for usefulness of application of genetic data in clinical settings for predicting the treatment response. In Asians, patients carrying IL28B low response alleles and genotypes could be identified for longer antiviral treatment. However, SVR of HCV infection is determined by multiple genetic loci. The practical utility of genetic data in treatment choice remains to be determined for IFN-based therapy.

In conclusion, IFN- λ s appear as critical functional immune response molecules that could be therapeutic target in the treatment of persistent HCV infections. Genetic data of IL28B SNPs may provide novel guidelines in determining optimal treatment duration with IFN-based therapies.

Materials and methods

Study subjects

This study recruited 728 patients from the Hepatology clinics of Linkou Medical Center, Chang Gung Memorial Hospital. Hepatology specialists confirmed the chronic HCV infection clinically and verified the diagnosis with liver biopsies in all examined patients. Two cohorts of patients with chronic hepatitis C were analyzed. Patients in cohort A were treated with INTRON plus ribavirin (126 patients, January 1988 to October 2001) and patients in cohort B were treated with pegylated IFN plus ribavirin (604 patients, November 2001 to December 2008). All patients were positive for anti-HCV (for >6 months) and for HCV-RNA. In addition, HCV from each patient was genotyped and the infections were verified with pathological findings before the enrolment for treatment. The patients in cohort A received INTRON

(IFN- α 2b, Schering Co., Kenilworth, NJ, USA) 5 MU three times a week and ribavirin 1200 mg per day. The cohort B patients received ribavirin 1200 mg per day and PEGINTRON (15 μ g kg⁻¹ week⁻¹, Peginterferon- α 2b, Schering Co.) or PEGASYS (180 μ g week⁻¹, Peginterferon- α 2a, Roche). For the purpose of this study, a total of 960 healthy blood donors (514 men and 446 women) were recruited following a questionnaire survey to exclude any donors with autoimmune diseases including rheumatoid arthritis, systemic lupus erythematosus, ankylosing spondylitis, and autoimmune thyroiditis, diabetes mellitus, viral hepatitis (HBV and HCV) infections and cardiovascular diseases. The age of healthy control donors ranges from 18 to 64 years old with a mean age of 40.3 \pm 10.7.

HCV infection assays

Clinical characteristics including liver biochemical test, HCV genotypes, HCV viral load, liver histology and therapeutic responses to the combination regimen (IFN- α plus ribavirin). The HCV antibody was tested by the Abbott AxSYM anti-HCV 3.0 (Abbott Laboratories, Abbott Park, IL, USA). The HCV RNA detection was carried out with Cobas Amplicor HCV v2.0 kit (Roche Diagnostics Co., Indianapolis, IN, USA; lower limit of detection of at concentrations of 50 IU ml⁻¹) and RNA was quantitated by the VERSANT HCV RNA 3.0 Assay (bDNA) (Bayer Diagnostics, Berkeley, CA, USA; lower limit of detection of at 615 IU ml⁻¹) or COBAS TaqMan HCV Test (TaqMan HCV; Roche Molecular Systems Inc., Branchburg, NJ, USA; lower limit of detection of at concentrations of 15 IU ml⁻¹). The baseline levels of HCV-RNA were determined by VERSANT HCV RNA 3.0 Assay in 669 patients and confirmed by Cobas Amplicor HCV v2.0 before January, 2008. Thereafter, COBAS TaqMan HCV Test was applied in 59 patients. HCV RNA level <2 MEQ (equal to 400 000 IU ml⁻¹) was defined as low viral load. HCV genotypes were analyzed using a genotype-specific probe-based assay in the 50 untranslated region.

Clinical SVR and liver biopsy

SVR was defined when serum HCV-RNA was undetectable at the end of therapy and at 24-week follow-up after the treatment. Non-response was designated when viral RNA reappears within 6 months after the cessation of treatment; or when viral load decreases <2 logs after 12-week treatment, or when viral RNA was detectable at the end of treatment. The liver biopsy was interpreted and graded by Ishak classification. In the analysis, we categorized fibrosis into cirrhosis (fibrosis score 5 or 6), severe fibrosis (fibrosis score 4) and mild fibrosis (fibrosis score 1 or 2 or 3).

Nucleic acid isolation

Anti-coagulated peripheral blood was obtained from healthy normal blood donors and HCV patients. Genomic DNA was isolated from EDTA anti-coagulated peripheral blood using the Puregene DNA isolation kit (Gentra Systems, Minneapolis, MN, USA) as previously described.⁶⁰

Taqman-based assay for the genotyping of SNPs

The oligonucleotide sequences flanking IL28B polymorphisms were designed as primers for Taqman allelic

discrimination assays. Allele-specific primers were labeled with a fluorescent dye (FAM or VIC) and used in the PCR reaction. Aliquots of the PCR products were genotyping using allele specific probe of SNPs on a real-time PCR Thermocycler (ABI, Foster City, CA, USA).

Statistical analysis

We carried out single-locus analyses of IL28B polymorphisms for 960 normal healthy controls and 728 patients with chronic HCV infection. Three χ^2 tests: the genotype test, the allele test and Cochran-Armitage trend test were performed, and associations with SNPs ($P < 0.05$) were identified using the SAS/Genetics software package release 8.2 (SAS Institute, Cary, NC, USA). For the analysis of risk genotypes/alleles, we used logistic regression models adjusted for age and sex and calculated P -values, ORs, and their 95% CIs. Linkage disequilibrium between marker loci was assessed and haplotype blocks were constructed using Haploview 4.1 (Broad Institute of MIT and Harvard; <http://www.broad.mit.edu/mpg/haploview>). For the markers within the same haplotype block, we used disease status (case vs control) and five clinical characteristics: body mass index, drug, HCV genotype, HCV RNA viral load and SVR as traits and tested for the haplotype-trait association utilizing SAS HAPLOTYPE procedure. To investigate the genetic association with clinical characteristics including body mass index, drug, HCV genotype, HCV RNA viral load and SVR for the case group, we controlled for each of these five characteristics and performed stepwise logistic regression analyses. Additionally, we examined the association of SVR with age on IFN therapy, gender, body mass index, HCV RNA viral load, HCV genotype, adherence and degrees of liver fibrosis in the chronic HCV infection patients using Student's t -test and χ^2 test. The 5% level of significance for P -values was used for all the analyses.

Conflict of interest

The authors declare no conflict interest.

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