

IL28B polymorphisms influence stage of fibrosis and spontaneous or interferon-induced viral clearance in thalassemia patients with hepatitis C virus infection

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Online Supplementary Appendix

Experimental procedures

The Taqman SNP genotyping assays (AB Foster City, CA, USA) use fluorogenic 5-nuclease chemistry to discriminate specific PCR products. PCR reactions were carried out on 10 ng of purified DNA in a 5 µL final volume containing 2.5 µL of Taqman Universal PCR master mix (2X), 0.125 µL of either *rs12979860* or *rs8099917* SNP genotyping assay stock solution (40X), and 2.375 µL of DNase free water, using an ABI Prism 9700 Thermocycler (ABI, Foster City, CA, USA). PCR conditions were one cycle at 50°C for 2 min and one cycle at 95°C for 10 min, followed by 40 cycles of denaturation at 92°C for 15 s, and annealing/extension at 60°C for 1 min.

Genotyping results were confirmed by direct sequencing of 8 randomly selected samples per genotype. For IL28 genomic amplification, the following primers were used: a) *rs12979860* (327 bp); 5'-AGCTCAGCGCCTCTTCCT-3' (forward) and 5'-CACAAAT CCCACCACGAGAC-3' (reverse); b) *rs8099917* (301 bp); 5'-TGTGCATATGTTTTCTGACTACCA-3' (forward) and 5'-GTT TCCTGCTGGGCCCTAA-3' (reverse).

The KASPar SNP Genotyping Method (KBioscience, Herts.,

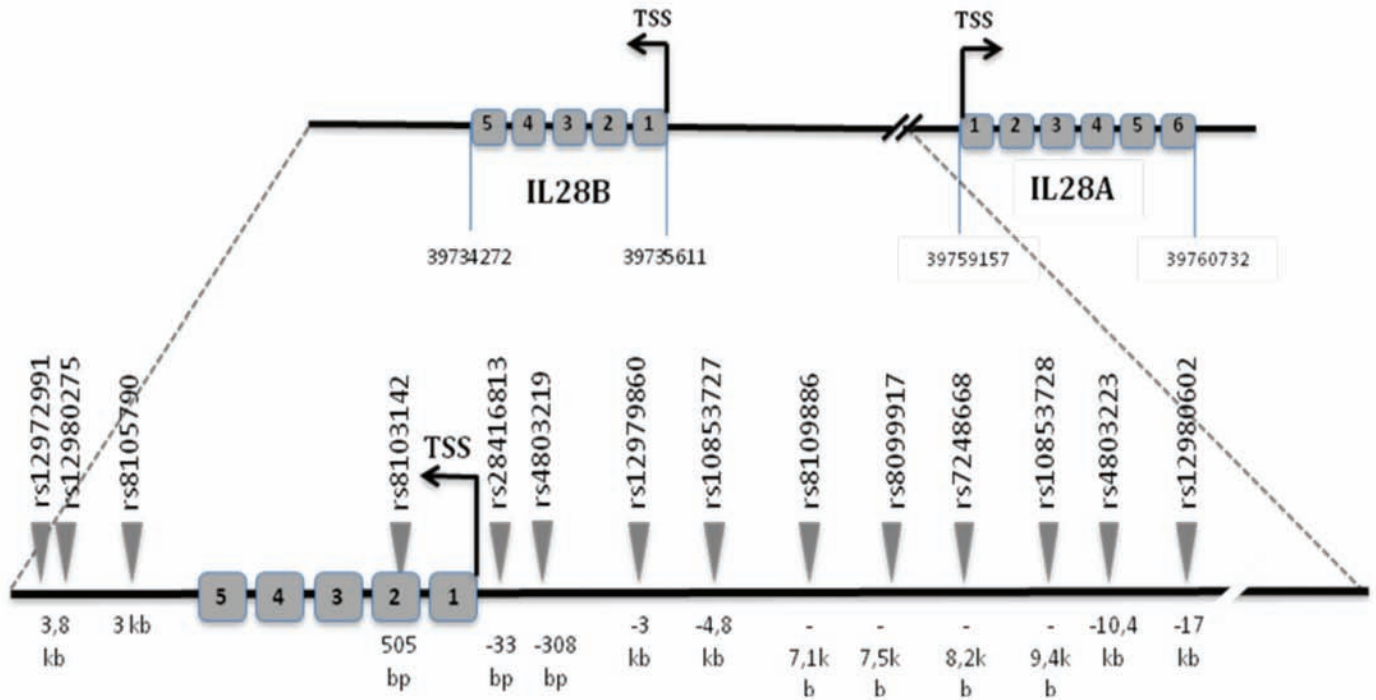
UK) uses a FRET-based system for SNP detection. Custom genotyping assays for the *rs12979860*, *rs12980275*, *rs8099917*, *rs12972991*, *rs8109886*, *rs4803223*, *rs12980602*, *rs8105790*, *rs8103142*, *rs28416813*, *rs4803219*, *rs7248668*, *rs10853727* and *rs10853728* were designed by submitting the SNP sequences to KBioscience, and carried out using an ABI Prism 9700 Thermocycler (ABI, Foster City, CA, USA). PCR reactions were carried out on 10 ng of purified DNA in a final volume of 5 µL (2.5 µL of 2X KASP reaction mix, 0.07 µL of SNP genotyping assay working stock solution, and 2.5 µL of DNase free water). The following thermal cycling conditions were used: an initial step at 95°C for 15 min to activate the Hot-Start polymerase; 10 cycles of touchdown PCR (denaturation at 94°C for 20 s, and annealing/extension from 65°C to 57°C for 1 min, with a decrease of -0.8°C per cycle); and 36 cycles of PCR (denaturation at 94°C for 20 s and annealing/extension at 57°C for 1 min). Genotyping calls were confirmed by direct sequencing of 6 randomly selected samples per genotype.

Sequencing was carried out for both allelic discrimination assays and KASPar FRET assays using the Dye Terminator Kit 3.1 method for both DNA strands in an ABI 3730 automated sequencer (ABI, Foster City, CA, USA).

References

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Human chr19 q13.2



Online Supplementary Figure S1. Genomic structure and positioning of 14 SNPs around the *IL28B* and *IL28A* genes (chr. 19 q13.2) that have shown a significant association with treatment-induced genotype 1 HCV clearance in genome-wide association studies (GWAS). The *rs12979860* and *rs8099917* SNPs were the top hit in the GWAS studies by Ge *et al.*,¹ Tanaka *et al.*,² Suppiah *et al.*,³ and Rauch *et al.*⁴ The *rs12980275*, *rs8099917*, *rs12972991*, *rs8109886*, *rs4803223* and *rs12980602* display different degrees of linkage disequilibrium with *rs12979860*, and their effects are, in the study by Ge *et al.*,¹ largely explained by *rs12979860*, including the *rs12980602*, located 6.0 Kb upstream from the translation starting site (TSS) of the *IL28A* gene and 17kb upstream from the TSS of the *IL28B* gene *rs28416813* (a G>C transition 33 bp upstream from the TSS of the *IL28B* gene). In addition, the nonsynonymous SNP *rs8103142* (amino-acid substitution Lys70Arg) was strongly associated with *rs12979860*, and its individual contribution to SVR could not be verified.¹ *rs12972991*, *rs12980275*, *rs8105790*, *rs8103142*, *rs28416813*, *rs4803219*, *rs8099917*, *rs7248668* and *rs10853728* showed strong associations with virological response in the GWAS study by Tanaka *et al.*² In the GWAS study by Suppiah *et al.*,³ in addition to the tag SNP, *rs8099917*, *rs12980275*, *rs8105790*, *rs8103142*, *rs10853727*, *rs8109886* were all significantly associated with SVR. *rs12972991*, *rs12980275*, *rs8105790*, *rs10853727*, *rs8109886*, *rs8099917*, *rs7248668*, and, to a lesser extent, *rs10853728*, *rs4803223* and *rs12980602* were associated with progression to chronic HCV infection in the GWAS study by Rauch *et al.*⁴ The *rs12979860* C/T allele distribution was shown to be associated with spontaneous clearance of hepatitis C virus (5) and the clinical course of chronic HCV hepatitis.

Online Supplementary Table S1. Correlation between single nuclear polymorphisms of IL28B locus and HCV-related outcomes.

IL28B SNPs	Major genotype	Spontaneous HCV clearance (98 of 245 patients, 40%)			Staging F0-F2 at liver biopsy (84 of 131 patients, 64.1%)			SVR after IFN therapy (30 of 89 genotype 1b patients, 33.7%)		
		Major genotype prevalence (%)	OR (95% - CI)	P	Major genotype prevalence (%)	OR (95% - CI)	P	Major genotype prevalence (%)	OR (95% - CI)	P
rs 18099917	T/T	63.2	1.89 (1.07 - 3.35)	0.028	58.1	3.68 (1.67 - 8.08)	0.001	57.3	4.25 (1.48 - 12.14)	0.007
rs1279860	C/C	43.8	2.31 (1.34 - 3.98)	0.003	36.4	3.41 (1.44 - 8.05)	0.005	33.3	3.81 (1.45 - 10.04)	0.007
rs 7248668	G/G	63.6	1.99 (1.12 - 3.52)	0.018	57.9	3.79 (1.74 - 8.24)	0.001	57.1	4.23 (1.48 - 12.02)	0.007
rs11881222	A/A	45.6	2.10 (1.22 - 3.59)	0.007	38.0	3.15 (1.36 - 7.25)	0.007	35.7	4.00 (1.52 - 10.47)	0.005
rs12980275	A/A	43.4	1.68 (0.98 - 2.88)	0.056	38.8	3.32 (1.44 - 7.64)	0.005	36.9	4.63 (1.75 - 12.23)	0.002
rs8113007	A/A	45.4	1.98 (1.16 - 3.39)	0.012	38.3	3.06 (1.32 - 7.06)	0.009	35.7	4.00 (1.52 - 10.47)	0.005
rs12972991	A/A	52.4	1.42 (0.83 - 2.42)	0.197	50.0	4.13 (1.86 - 9.16)	< 0.001	50.0	5.00 (1.81 - 13.75)	0.002
rs8109886	A/A	23.2	0.84 (0.45 - 2.58)	0.606	23.1	0.60 (0.25 - 1.42)	0.251	22.6	5.66 (1.20 - 26.61)	0.028
rs4803223	A/A	70.4	2.04 (1.10 - 3.79)	0.024	66.4	3.16 (1.43 - 7.00)	0.004	71.1	8.66 (1.86 - 40.26)	0.006
rs12980602	T/T	59.2	1.93 (1.11 - 3.36)	0.020	54.5	2.57 (1.20 - 5.48)	0.014	58.1	7.44 (2.28 - 24.27)	0.001
rs10853727	T/T	78.4	0.81 (0.42 - 1.56)	0.542	77.5	0.79 (0.32 - 1.95)	0.612	75.9	1.25 (0.42 - 3.71)	0.685
rs28416813	C/C	45.2	2.33 (1.36 - 4.01)	0.002	37.2	2.98 (1.29 - 6.88)	0.010	34.5	3.46 (1.32 - 9.02)	0.011
rs10853728	G/G	46.3	1.62 (0.95 - 2.78)	0.076	52.1	0.69 (0.33 - 1.45)	0.334	51.2	0.60 (0.24 - 1.51)	0.282
rs8105790	T/T	63.2	1.88 (1.07 - 3.32)	0.028	57.9	3.79 (1.74 - 8.24)	0.001	58.3	3.93 (1.38 - 11.18)	0.010