Genetic variation in *IL28B* and spontaneous clearance of Hepatitis C Virus

Supplementary information

I. Analysis adjusting by homozygosity for *KIR2DL3/HLA-C* group 1

We previously showed a protective effect of homozygosity for the compound genotype *KIR2DL3/HLA-C* group 1 in injection drug users[1]. We therefore adjusted for the presence of this protective compound genotype in our analyses. After adjustment, the association with the rs12979860 C allele and HCV clearance remained unaltered (data not shown). In the Khakoo et al. study, the protective effect was observed only among injection drug users, who likely received low-titer HCV inocula, but not among hemophiliacs who are likely to have received relatively large viral titer inocula. The interpretation for this difference was that the influence of natural killer (NK) cell activity, as modulated by the KIR2DL3 receptor/ HLA-C ligand combination, was great enough to affect outcome when the individuals were exposed to low levels of virus, but that the NK cell response could not contain a large bolus of viral inoculum, such as that received by hemophiliacs. We therefore tested whether there were any differences in the effect of the protective rs12979860 C allele in infection acquired by blood products (high titre inoculum) versus that acquired by injection drug use (low titre inoculum), but no significant differences were observed between the two groups (data not shown).

II. Analysis of the effect of rs12979860 on HCV viral load

HCV viral load data was available for 404 individuals from MHCS, HGDS and ALIVE. One blood sample from each individual was assessed for HCV RNA by a

bDNA assay (see II above). Viral load levels were $\log_{10}$ transformed and the values were correlated to rs12979860 genotype in all racial groups and whites and blacks separately. A regression model ($\log_{10}$VL as dependent variable and genotype as independent variable) was performed using SAS PROC REG. In the model, genotype data were used in the form of ordered categories, where CC=2, CT=1 and TT=0.

Table SI: HBV does not confound the effect of rs12979860 genotype on outcome of HCV infection

				Adjusted by cohort and ethnicity	
All subjects, genotype	**Frequency of clearance, %(N)**	**Frequency of persistence, %(N)**	**Comparison**	**OR (95% CI)**	**P value**
TT	23.4 (37)	76.6 (121)	CC vs TT	0.29 (0.18-0.47)	$4x10^{-7}$
CT	29.5 (124)	70.5 (297)	CC vs CT	0.35 (0.25-0.48)	$4x10^{-11}$
CT+TT	28 (161)	72 (418)	CC vs CT+TT	0.33 (0.25-0.45)	$3x10^{-13}$
CC	53 (227)	47 (202)			
All subjects, genotype				Adjusted by cohort, ethnicity and HBV status	
TT	21.8 (32)	78.2 (115)	CC vs TT	0.29 (0.18-0.47)	$8x10^{-7}$
CT	25.6 (98)	74.4 (285)	CC vs CT	0.34 (0.25-0.47)	$9x10^{-11}$
CT+TT	24.5 (130)	75.5 (400)	CC vs CT+TT	0.33 (0.24-0.44	$7x10^{-13}$
CC	47.9 (178)	52.1 (194)			
White subjects, genotype				Adjusted by cohort and HBV status	
TT	29.2 (14)	70.8 (34)	CC vs TT	0.51 (0.26-1.02)	0.06
CT	22.3 (51)	77.7 (178)	CC vs CT	0.34 (0.23-0.51)	$2x10^{-7}$
CT+TT	23.5 (65)	76.5 (212)	CC vs CT+TT	0.37 (0.25-0.54)	$2x10^{-7}$
CC	44.8 (126)	55.2 (155)			
Black subjects, genotype				Adjusted by cohort and HBV status	
TT	19.3 (17)	80.7 (71)	CC vs TT	0.19 (0.09-0.41)	$2x10^{-5}$
CT	32.3 (42)	67.7 (88)	CC vs CT	0.40 (0.21-0.76)	0.01
CT+TT	27.1 (59)	72.9 (159)	CC vs CT+TT	0.31 (0.17-0.57)	$2x10^{-4}$
CC	55.2 (32)	44.8 (26)			

OR = odds ratio; CI = confidence interval

Table S2: HIV does not confound the effect of rs12979860 genotype on outcome of HCV infection

				Adjusted by cohort and ethnicity	
All subjects, genotype	**Frequency of clearance, %(N)**	**Frequency of persistence, %(N)**	**Comparison**	**OR (95% CI)**	**P value**
TT	23.4 (37)	76.6 (121)	CC vs TT	0.29 (0.18-0.47)	$4x10^{-7}$
CT	29.5 (124)	70.5 (297)	CC vs CT	0.35 (0.25-0.48)	$4x10^{-11}$
CT+TT	28 (161)	72 (418)	CC vs CT+TT	0.33 (0.25-0.45)	$3x10^{-13}$
CC	53 (227)	47 (202)			
HIV positive subjects, genotype				Adjusted by cohort and ethnicity	
TT	12.5 (5)	87.5 (35)	CC vs TT	0.12 (0.04-0.36)	$2x10^{-4}$
CT	24 (23)	76 (73)	CC vs CT	0.22 (0.11-0.45)	$2x10^{-5}$
CT+TT	20.6 (28)	79.4 (108)	CC vs CT+TT	0.20 (0.10-0.37)	$9x10^{-7}$
CC	52.2 (47)	47.8 (43)			
HIV negative subjects, genotype				Adjusted by cohort and ethnicity	
TT	27.1 (32)	72.9 (86)	CC vs TT	0.37 (0.21-0.63)	$3x10^{-4}$
CT	31.1 (101)	68.9 (224)	CC vs CT	0.39 (0.28-0.56)	$2x10^{-7}$
CT+TT	30 (133)	70 (310)	CC vs CT+TT	0.38 (0.28-0.54)	$2x10^{-8}$
CC	53.1 (180)	46.9 (159)			

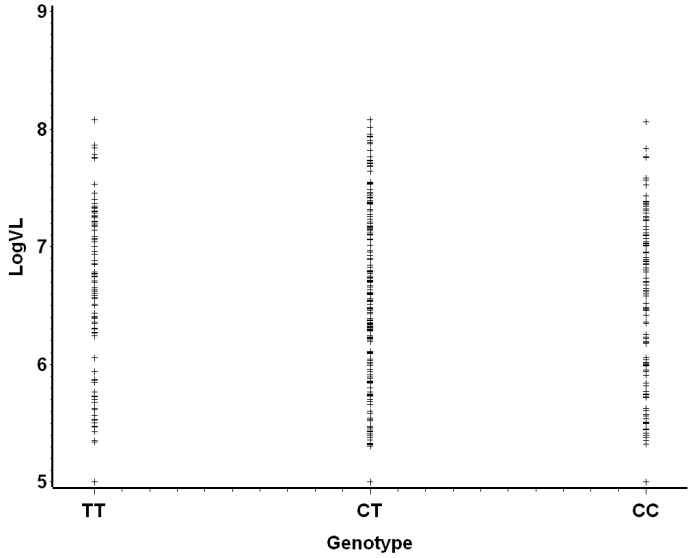
OR = odds ratio; CI = confidence interval

Table S3: There is no evidence for bias as a consequence of the lack of matching in some cohorts on the effect of rs12979860 genotype on outcome of HCV infection

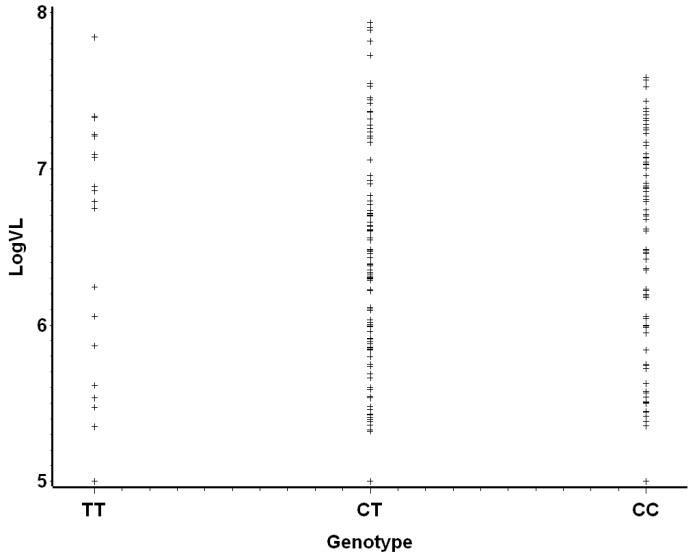
				Adjusted by cohort and ethnicity	
All subjects, genotype	**Frequency of clearance, %(N)**	**Frequency of persistence, %(N)**	**Comparison**	**OR (95% CI)**	**P value**
TT	23.4 (37)	76.6 (121)	CC vs TT	0.29 (0.18-0.47)	$4x10^{-7}$
CT	29.5 (124)	70.5 (297)	CC vs CT	0.35 (0.25-0.48)	$4x10^{-11}$
CT+TT	28 (161)	72 (418)	CC vs CT+TT	0.33 (0.25-0.45)	$3x10^{-13}$
CC	53 (227)	47 (202)			
Matched cohorts, genotype				Adjusted by cohort and ethnicity	
TT	22.4 (30)	77.6 (104)	CC vs TT	0.28 (0.16-0.48)	$4x10^{-6}$
CT	25.9 (78)	74.1 (223)	CC vs CT	0.33 (0.23-0.48)	$7x10^{-9}$
CT+TT	24.8 (108)	75.2 (327)	CC vs CT+TT	0.31 (0.22-0.45)	$1x10^{-10}$
CC	48.3 (124)	51.7 (133)			
Unmatched cohorts, genotype				Adjusted by cohort and ethnicity	
TT	29.2 (7)	70.8 (17)	CC vs TT	0.34 (0.11-1.02)	0.05
CT	38.3 (46)	61.7 (74)	CC vs CT	0.40 (0.23-0.71)	$2x10^{-3}$
CT+TT	36.8 (53)	63.2 (91)	CC vs CT+TT	0.40 (0.23-0.68)	$7x10^{-4}$
CC	59.9 (103)	40.1 (69)			

OR = odds ratio; CI = confidence interval

A. All subjects



B. White subjects



C. Black subjects

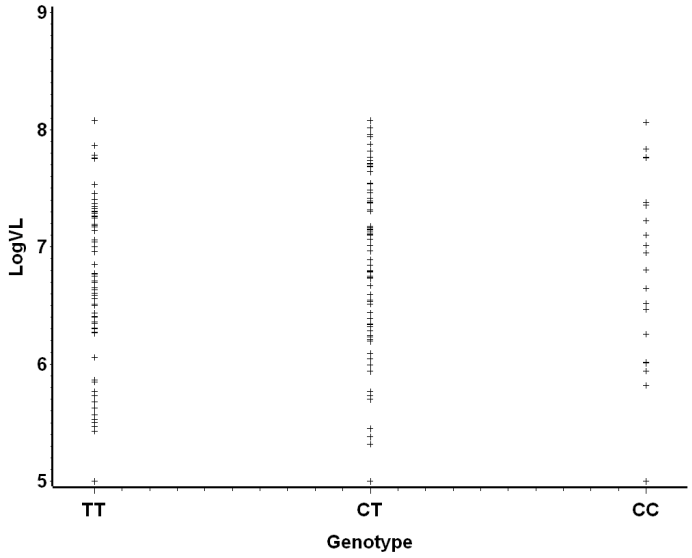


Figure S1. The effect of rs12979860 genotype on HCV viral load. A) All patients, B) white subjects, C) Black subjects. The results indicate that there is no correlation between HCV viral load and rs12979860 genotype (R^2 = 0.0015, 0.0006, 0.0055 for all subjects, whites and blacks respectively).

References

1 Khakoo, S.I. *et al.*, HLA and NK cell inhibitory receptor genes in resolving hepatitis C virus infection. *Science* 305 (5685), 872-874 (2004).