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Genome-Wide Association Study Identifies Variants Associated with Progression of Liver Fibrosis from HCV Infection

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Abstract

BACKGROUND & AIMS—Polymorphisms in *IL28B* were shown to affect clearance of hepatitis C virus (HCV) infection in genome-wide association (GWA) studies. Only a fraction of patients with chronic HCV infection develop liver fibrosis, a process that might also be affected by genetic factors. We carried out a 2-stage GWA study of liver fibrosis progression related to HCV infection.

METHODS—We studied well-characterized HCV-infected patients of European descent who had liver biopsies before treatment. We defined various liver fibrosis phenotypes on the basis of Metavir scores, with and without taking the duration of HCV infection into account. Our GWA analyses were conducted on a filtered primary cohort of 1161 patients using 780,650 single nucleotide polymorphisms (SNPs). We genotyped 96 SNPs with *P*-values<5×10^{-5} from an independent replication cohort of 962 patients. We then assessed the most interesting replicated SNPs using DNA samples collected from 219 patients who participated in separate GWA studies of HCV clearance.

RESULTS—In the combined cohort of 2342 HCV-infected patients, the SNPs rs16851720 (in the total sample) and rs4374383 (in patients that received blood transfusions) were associated with fibrosis progression ($P_{combined}$=8.9×10^{-9} and 1.1×10^{-9}, respectively). The SNP rs16851720 is located within *RNF7*, which encodes an antioxidant that protects against apoptosis. The SNP rs4374383, together with another replicated SNP, rs9380516 ($P_{combined}$=5.4×10^{-7}), were linked to the functionally related genes *MERTK* and *TULP1*, which encode factors involved in phagocytosis of apoptotic cells by macrophages.

CONCLUSIONS—Our GWA study identified several susceptibility loci for HCV-induced liver fibrosis; these were linked to genes that regulate apoptosis. Apoptotic control might therefore be involved in liver fibrosis.

Keywords

genetic analysis; risk factors; cirrhosis; liver disease

Introduction

End-stage chronic hepatitis C is the leading cause of liver transplantation in developed countries and more than 350,000 people die from HCV-related liver diseases each year.[1] The natural course of chronic HCV infection is characterized by high levels of inter-individual variation in disease progression.[2] Most subjects never develop cirrhosis, but some may develop severe fibrosis in less than 20 years. A number of host demographic and clinical characteristics, as well as viral factors, have been associated with the development of HCV-related liver fibrosis.[2–4] However, these factors account for only a small proportion of the variability in the rate of liver fibrosis development, which overall remains unpredictable. There is accumulating evidence to suggest that host genetic factors are involved, although these factors remain largely unknown.[2,5] A number of candidate gene approaches have been used in attempts to identify variants influencing the development of liver disease in HCV-infected patients, but most of these studies produced results that were not consistently replicated.[5,6] Two related studies[7,8] investigating ~25,000 putative functional SNPs identified a panel of SNPs predicting the risk of developing cirrhosis; this panel requires validation in prospective studies.[8] A recent study of 36 candidate genes, related to the fibrogenesis/fibrolysis process, identified a single cluster of variants of the *IFNGR2* gene associated with progression to severe fibrosis.[9] The results of these studies are interesting, but their approach may have prevented identification of genes strongly associated with liver fibrosis present in parts of the genome not tested.

Genome-wide association (GWA) studies provide a broader and unbiased approach for the discovery of genetic factors involved in disease susceptibility.[10] For example, GWA studies identified a SNP cluster in the *IL28B* gene with a major effect on HCV clearance, either treatment-induced or spontaneous, whereas this gene had never previously been implicated in HCV infection.[11] A recent candidate gene study found that *IL28B* alleles associated with poor HCV clearance had a protective effect against liver inflammation and fibrosis.[12] No GWA study has yet explored genetic susceptibility to liver fibrosis in patients with chronic HCV infection. Several GWA studies of liver disease-related traits demonstrated a role for a non-synonymous variant of the *PNPLA3* gene, rs738409 (I148M), in the development of nonalcoholic and alcoholic fatty liver disease and associated disease severity.[13–15] The same *PNPLA3* risk allele was recently associated with steatosis and liver fibrosis in patients with chronic HCV infection.[16,17] In this study, we carried out a two-stage GWA study (primary screen followed by a replication study) in a combined cohort of 2,342 well characterized HCV-infected patients, to identify genetic factors influencing the development of HCV-related liver fibrosis.

Patients & Methods

Patient Subjects

The sample used for the primary screen combined data from two cohorts of adult patients of European descent from France and Switzerland with chronic HCV infection. We retained only patients who had liver biopsy before treatment. The French cohort (ANRS Genoscan study group) included patients from the hepatology units of several hospitals in Paris and Marseilles; the inclusion criteria applied, including no co-infection by HIV or HBV, have been described elsewhere.[9] The Swiss Hepatitis C Cohort Study (SCCS) is a multicenter study of HCV-infected patients enrolled at eight major Swiss hospitals and the affiliated local centers. SCCS patient selection and data collection have also been described elsewhere,[3,18] and patients with known HIV or active HBV co-infection were excluded for the present study. In total, 1,223 patients (490 from the French cohort and 733 from the SCCS) were eligible for genetic analyses. We studied three additional cohorts of European-descent adult patients with chronic HCV infection and not co-infected with HIV or HBV,

with the aim of replicating the principal signals obtained in the primary cohorts. All 962 patients included in these cohorts had biopsy before treatment (Supplementary Table 1). A first cohort of 64 US patients was recruited at the Weill-Cornell Medical Center in New York, and a second sample of 256 French patients was recruited from different hospitals in Marseilles. The third replication cohort included 642 patients recruited from centers in Australia, Germany, the United Kingdom and Italy, as described elsewhere.[19,20] Finally, the seven signals showing evidence of true replication in the cohort combining primary and replication cohorts were also tested in an additional independent sample of Australian patients consisting of the primary cohort of a previous GWA study of response to hepatitis C treatment.[19] After excluding Australian patients with missing phenotype data, 219 individuals were kept for this analysis (Supplementary Table 1). Clinical risk factors, history of HCV acquisition and of alcohol consumption (assessed using time-line follow back interview) were recorded in the corresponding cohorts through face-to-face interviews conducted by physicians trained in addiction problems. The sampling of all the cohorts was approved by the appropriate institutional review boards, and written informed consent was obtained from all patients.

Determination of Liver Fibrosis Phenotypes

The stage of liver fibrosis in patients with chronic HCV infection was determined by examination of a liver biopsy specimen obtained before treatment, with quantification according to the Metavir score, on a five-point scale from F0 to F4.[21] Fibrosis was assessed by experienced pathologists working in the local expert liver centers corresponding to the place of collection, and all biopsies were considered as interpretable by each local pathologist. Metavir scoring system is validated since many years and highly reproducible for pathologists specialized in liver disease.[22] For each patient, we used the information obtained from a single biopsy, referred to as the reference biopsy. For patients who had undergone several biopsies, the reference biopsy was defined as follows: (i) the most recent biopsy obtained in the absence of treatment for patients who had not spontaneously developed fibrosis, i.e. all biopsy results without treatment were F0 or F1; (ii) the biopsy with the highest Metavir score for patients who had developed fibrosis, i.e. at least one biopsy result ≥F2; the earliest biopsy was selected if there were several biopsies with the highest score. We used three different approaches to define liver fibrosis phenotype on the basis of the Metavir score for the reference biopsy. The first of these approaches was a classic case/control approach in which F0-1 patients were considered as controls and F3-4 patients, as cases. Patients with an intermediate Metavir score (F2) were discarded for this analysis, which was thus a *binary F0-1/F3-4* phenotype analysis. Within this approach, we also used a more extreme definition of the phenotypes, in which we considered only F0 patients as controls and F4 patients as cases (*binary F0/F4* phenotype analysis). In the second approach, we used survival analysis techniques to take into account the individual duration of infection in the analysis. Failure (F3-4 or only F4) and censored (F0-1 or only F0) events were defined as in the case/control study, and the duration of infection was estimated from the presumed year of HCV acquisition to the year in which the biopsy was carried out. The estimated date of HCV acquisition was obtained using the first reported major event at risk among blood transfusion, drug use (the first year of injecting drug use (IDU) was used as the starting point), accidental needle stick or other invasive medical procedures. In this analysis, the phenotype is denoted *duration F0-1/F3-4* or *duration F0/F4*. In the third approach, liver fibrosis progression was considered as a quantitative phenotype, fibrosis progression rate (FPR), corresponding to the ratio of Metavir score to the estimated duration of infection in years (Metavir units per year).[23,24] Patients with a F2 Metavir score were included in this analysis. Given the distribution of FPR, this phenotype was log-transformed and then inverse normal quantile-transformed in each cohort before statistical analysis. The resulting phenotype, denoted as *QTF* phenotype, was further standardized such

that the differences in mean QTF rates between genotypes for a given SNP could be expressed in standard deviation units, referred to as standardized Metavir units (SMUs). Posterior to the selection of the 96 followed-up SNPs (see next sections), we analyzed our primary data by modeling transition probabilities between Metavir stages using a Markov model, described in Supplementary Methods. This approach was not further considered, because it provided no additional association signals with *P* values<5×10^{-6} in our primary cohort, with respect to analyses using the three other phenotype definitions.

Genotyping and Imputation Procedures

Details of genotyping methods, quality controls and imputation procedures are provided in the Supplementary Methods. Briefly, the French and Swiss primary cohorts were genotyped for ~350,000 SNPs and ~1,000,000 SNPs, respectively, using Illumina HumanCNV370-Duo and Human1M-Duo beadchips (Illumina, San Diego, USA). Quality-control filtering of SNPs resulted in a total of 325,624 and 912,765 high-quality SNPs, respectively. Genotype imputation was performed in the French primary cohort, using the Swiss cohort as a template. This procedure yielded a total of 780,650 high-quality genotyped or imputed SNPs, which were used for all analyses. Quality-control filtering was also carried out for individuals, and 1,161 filtered individuals were used for GWA analyses (Table 1). The 962 subjects of the replication cohorts (Supplementary Table 1) were genotyped for 96 followed-up SNPs by Illumina GoldenGate genotyping with VeraCode technology (Illumina), as well as our French primary cohort for 33 out of the 96 SNPs that were initially imputed in this cohort. Eighty-seven high-quality SNPs were tested for replication. Among the seven SNPs showing evidence for true replication, two SNPs, rs16851720 and rs4374383, were not present in the GWA cohort of 219 Australian patients, genotyped by the Illumina Infinium HumanHap300 or the CNV370-Quad genotyping BeadChips (Illumina). We could impute these two SNPs with a high accuracy of 0.996 and 0.984, respectively (Supplementary Methods).

Statistical Analyses

For liver fibrosis considered *as binary* phenotypes, we used Fisher's exact test and logistic regression. For *duration* phenotypes, we used a Cox model considering estimated age at infection as the starting point and the first biopsy showing severe fibrosis (failure time) or the last biopsy showing an absence of severe fibrosis in the absence of treatment (censored time) as the endpoint. Logistic regression and Cox model analyses were used for stratified and multivariate adjusted analyses, with sex, HCV genotype, age at infection, alcohol consumption and/or mode of HCV acquisition as covariates. For the *QTF* phenotype, linear regression analysis was performed. All statistical analyses were carried out with procedures implemented in SAS software v.8.2 (SAS Institute, Cary, North Carolina, USA), R software (http://www.R-project.org/), Matlab (www.mathworks.com) and PLINK.[25] The power of this study was estimated for the *binary F0-1/3–4* and the *duration F0-1/3–4* phenotypes, as described in Supplementary Methods.

As we conducted several analyses, using different phenotypes, genetic models and stratified samples, we estimated the effective number of tests in our study by a method detailed in the Supplementary Methods. Briefly, we estimated that the analyses conducted in the whole sample corresponded to a total number of ~3 million effective tests. Adjusted and stratified analyses were performed only on the *binary* and *duration F0-1/F3-4* phenotypes, for the 140,797 SNPs giving *P*-values<0.05 in the analyses conducted on these two phenotypes in the whole sample, taking eight strata into account: age at infection>20 years, age at infection<20 years, male, female, contamination by transfusion, contamination by IDU, HCV genotype=1 and HCV genotype≠1. The number of effective tests for these stratified analyses was estimated at ~1.6 million, giving a total of ~4.9 million independent tests for

our entire GWA analysis. We therefore considered *P*-values<10^{-8} ($0.05/4.9\times10^{6}$) to indicate genome-wide significance in our study.

Results

Genome-wide analyses in the primary cohort

GWA analyses were conducted on a filtered primary cohort of 1,161 HCV-infected patients (Table 1), with a total of 780,650 SNPs. We first used the *binary F0-1/F3-4* phenotype of liver fibrosis and assumed an additive genetic model. No significant deviations from expectations were observed on quantile-quantile plots before and after correction for population stratification (Supplementary Figure 1), attesting to an absence of difference in ancestry between cases and controls (genomic inflation factor λ=1.013). In addition, none of the first 10 principal components of ancestry was associated with case-control status (Supplementary Figure 2). We therefore carried out all subsequent analyses without adjustment for population stratification.

Figure 1A shows the Manhattan plot obtained for analysis of the *binary F0-1/F3-4* phenotype, for the best among the three genetic models tested (additive, recessive, dominant). *P*-values<10^{-6} were obtained for two SNPs on chromosomes 2 and 8 (Supplementary Table 2). When restricting our cohort to the most extreme Metavir scores (*binary F0/F4* phenotype), a single locus on chromosome 2, tagged by four SNPs in strong linkage disequilibrium (LD), achieved a *P*-value<10^{-6} (Supplementary Figure 3A and Supplementary Table 2). We then carried out GWA analyses taking into account the estimated duration of infection in 1,064 subjects for whom follow-up information was available. Two SNPs on chromosomes 11 and 18 provided *P*-values<10^{-6} when considering the *duration F0-1/F3-4* phenotype (Figure 1B and Supplementary Table 2). When restricting the analysis to the *duration F0/F4* phenotype, a *P*-value<10^{-6} was obtained for one SNP on chromosome 6 (Supplementary Figure 3B and Supplementary Table 2). Finally, the GWA analysis of the *QTF* phenotype identified a single SNP on chromosome 3 with a *P*-value<10^{-6} (Figure 1C and Supplementary Table 2). For all phenotypes, 45 additional independent signals gave *P*-values<5×10^{-5} (Supplementary Table 3).

All the classical risk factors for fibrosis development available in our sample (i.e. sex, alcohol consumption, HCV genotype, HCV mode of acquisition and age at infection) were significantly and independently associated with fibrosis progression in our sample, in multivariate logistic regression analysis (Supplementary Table 4). Multivariate analyses of our *binary* and *duration F0-1/F3-4* phenotypes adjusted for these factors did not substantially change our previous results (Supplementary Table 3). We also performed adjusted and stratified analyses for the *binary* and *duration F0-1/F3-4* phenotypes, using the 140,797 SNPs that achieved a *P*-value<0.05 in one of these two analyses. These analyses were performed taking into account binarized covariables such as sex (male/female), HCV genotype (1/others) or mode of acquisition (blood transfusion/IDU). In analyses with the *duration F0-1/F3-4* phenotype, six SNPs gave *P*-values<10^{-6}, including three with *P*-values<10^{-7} (Supplementary Table 2). In total, 38 additional independent signals gave *P*-values<5×10^{-5} (Supplementary Table 3).

Replication study

In the second phase, we genotyped a total of 96 independent SNPs presenting a *P*-value<5×10^{-5} in one of our previous GWA analyses (Supplementary Table 3) in a replication cohort of 962 HCV-infected patients (Supplementary Table 1). Eighty-seven of these SNPs satisfying the quality-control filters were tested for association. Evidence for true replication (using the same model as in the primary analysis) at the 0.05 level was

obtained for seven SNPs (Table 2). We further tested these seven SNPs in an additional filtered cohort of 219 Australian patients with liver biopsy data (Supplementary Table 1), who were previously genotyped for ~310,000 SNPs on Illumina beadchips in a GWA study of response to chronic hepatitis C treatment.[19] Out of these seven SNPs, two (rs16851720 and rs4374383) were not present on the arrays, and were successfully imputed in this Australian cohort (Patients & Methods).

The combined analysis of these seven SNPs in the total sample of 2,342 patients identified two SNPs (Table 2) with *P*-values below our estimated genome-wide threshold for significance of 10^{-8} (see Patients & Methods for details). Tests of heterogeneity across primary and replication cohorts were not significant for either of these SNPs (Table 2). SNP rs16851720 provided a combined *P*-value=8.9×10^{-9} in the whole sample, in the analysis of the *QTF* phenotype (additive for C, difference between genotypes of 0.23 SMU (0.15–0.31); Figure 2A). This intronic SNP is located in *RNF7* (Supplementary Figure 4A), encoding a redox-inducible antioxidant protein that protects against apoptosis.[26] The second SNP was rs4374383, with *P*-value=1.1×10^{-9} in blood-transfused patients in analysis of the *duration F0-1/F3-4* phenotype (recessive for minor allele A, hazard ratio of developing liver fibrosis (HR) for AA vs.AG/GG=0.18 (0.09–0.36); Figure 2B). We found that this association was independent of viral genotypes (data not shown) although blood-transfused patients are more often infected with HCV genotypes 1 and 2. SNP rs4374383 is intronic in the *MERTK* gene (Supplementary Figure 4B), a member of the three TAM receptor tyrosine kinases which are involved in the regulation of inflammatory responses.[27]

Out of the five remaining replicated SNPs, two were improved or unchanged by the addition of the Australian cohort (Table 2). One was rs2629751 in analysis of the *binary F0/F4* phenotype (P=1.4×10^{-7}; recessive for G, OR=7.10 (2.94–20.74); Figure 2C), which is intronic in the *GLT8D2* gene. The other signal was identified using the *duration F0-1/F3-4* phenotype in men at SNP rs9380516 (P=5.4×10^{-7}; recessive for T, HR=4.53 (2.78–7.39); Figure 2D), which is located 21 kb downstream from the *TULP1* gene. The last three signals (SNPs rs883924, rs7800244 and rs6485480) were clearly less significant when the data for the Australian cohort were included in the analysis (Table 2). Consistent with this observation, tests of heterogeneity across cohorts were close to significance (*P*=0.06) and significant (*P*=0.01) for rs7800244 and rs6485480, respectively (Table 2).

Finally, we assessed, in our primary cohort, the association of liver fibrosis with a number of variants reported in large association studies (GWA studies or investigations of a large number of genes) to be associated with either liver fibrosis-related phenotypes in non HCV patients or other HCV-related phenotypes (Supplementary Table 5). Using the same methods as two previous studies partly overlapping with our primary cohort, we found that rs9976971 in *IFNGR2*[9] and rs8099917 in *IL28B*[12] were associated with liver fibrosis, with *P*-values of 0.009 and 0.018, respectively. We found no significant effect of variants reported to be associated with liver fibrosis in Caucasian patients with chronic HCV infection,[7,8] with liver fibrosis in Spanish patients with non-alcoholic fatty liver disease[28] or with the development of hepatocellular carcinoma in HCV-infected Japanese patients.[29,30] However, we found that rs738409 in *PNPLA3* was associated with liver fibrosis in our primary cohort, in analyses with the *binary F0-1/F3-4* phenotype (additive model for allele G, *P*=1.3×10^{-4}, OR=1.21 (1.10–1.34)). The non-synonymous *PNPLA3* variant rs738409 has been shown to be associated with several liver-related traits in GWA studies,[13–15] and with liver steatosis and fibrosis in patients chronically infected with HCV in studies based on candidate gene approaches.[16,17,31]

Discussion

We report the first GWA study investigating liver fibrosis progression in a large sample of more than 2,300 HCV-infected patients of European descent. For all patients, liver biopsy data were obtained before treatment. We defined several fibrosis phenotypes on the basis of histological findings, because the Metavir system grading is not linear,[21] and because we wished to investigate various and complementary aspects of the fibrosis process. In this context, we also sought to use an additional, more sophisticated approach, involving a Markov model of the probability of transition between Metavir stages (Supplementary Methods). This approach provided no additional signals with P values$<5\times10^{-6}$ for our primary cohort (data not shown). Overall, our analyses covered a large panel of liver fibrosis phenotypes in a well characterized sample of HCV-infected patients, and identified four replicated signals including two that were significant at the genome-wide level in the combined cohort.

Gene ontology enrichment analysis of our four most interesting signals revealed significant enrichment for two categories, “photoreceptor outer segment” and “phagocytosis” (P=5×10^{-4} and P=5×10^{-3}, respectively, after Bonferroni correction; Supplementary Methods). This result is explained by two SNPs: rs4374383, our most significant signal, and rs9380516, located within or close to *MERTK* and *TULP1*, respectively. Interestingly, rs4374383 is in strong LD (r^2>0.9, measured using HapMap phase II data in the CEU European-descent population) with two *MERTK* non-synonymous SNPs, rs7604639 and rs3811635, and rs9380516 is in strong LD (r^2=0.9 in CEU) with an intronic SNP of *TULP1*, rs9296155. Although Mendelian defects of these two genes affect only the retina,[32–34] both genes have a broader spectrum of expression, with *TULP1* expressed in the fetal liver[35] and *MERTK* expressed in macrophages.[27] In addition, the MERTK and TULP1 proteins have been shown to interact during the phagocytosis of apoptotic cells.[36] In particular, mice lacking specifically the murine homolog of *MERTK* display a deficiency in the clearance of apoptotic thymocytes by macrophages.[37] Interestingly, the clearance of apoptotic debris by phagocytosis can directly stimulate fibrogenesis.[38,39] Hepatic stellate cells (HSC) may play a key role in this process, as they have phagocytic functions, including the NADPH oxidase activity required for the production of reactive oxygen species (ROS).[40] HSC are also found in close proximity to hepatocytes, a key source of apoptotic debris during liver injury.[41] Overall, our results suggest a possible relationship between the clearance of apoptotic cells through phagocytosis and liver fibrosis.

The second signal significant at the genome-wide level was that for SNP rs16851720 in the whole sample. The effect of this SNP on fibrosis progression rate (FPR) was clearly additive in our combined cohort (Figure 2A). If FPR means were calculated directly as a function of rs16851720 genotypes, we found that each A risk allele increased the rate of liver fibrosis progression by 0.033 Metavir units/year, corresponding to ~1 Metavir unit over a 30-year period. This SNP is located in the first intron of *RNF7*, which is also known as *SAG* (sensitive to apoptosis gene). RNF7 is a cell-protecting molecule that acts as an antioxidant, inhibiting the apoptosis induced by metal ions and ROS,[26] thereby preventing DNA damage.[42] In this context, it is interesting to note that HCV inhibits host DNA damage repair through ROS production.[43] The induction of these processes, including high levels of ROS production in particular, in HCV-infected cells leads to hepatocyte apoptosis and HSC activation, and may also contribute to the development of liver fibrosis[44] through mechanisms described above.

In conclusion, for the whole sample, we found a genome-wide significant effect of the SNP rs16851720 on liver fibrosis, and confirmed the role of the *PNPLA3* non-synonymous I148M variant. We also found some interesting additional signals, including one genome-

wide significant, in more refined subsamples, which require further investigation. Overall, our findings suggest that several variants in a number of genes with rather modest effects (in the whole sample) and/or more complex effects (e.g. limited to a specific subsample) may be involved in genetic susceptibility to liver fibrosis secondary to chronic HCV infection. They also point out the role of apoptosis potentially providing new insights into the mechanisms underlying liver fibrosis development.[41,45]

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

E. P. and Z. K. contributed equally to the work. P.-Y. B. and L. Abel jointly directed the work. The members of the different study groups are listed in the Supplementary Material. We warmly thank all the study subjects. We thank all members of both branches of the laboratory of Human Genetics of Infectious Diseases for fruitful discussion. We are grateful to Alain Dessein (INSERM-UMR 906/Université de la Méditerranée, Marseilles), Marija Zeremski and Ray Peterson (Division of Gastroenterology and Hepatology, Weill-Cornell Medical College, New-York) for helping to collect the data.

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Abbreviations

HCV	hepatitis C virus
GWA	genome-wide association
SNP	single nucleotide polymorphism
IDU	injecting drug use
CI	confidence interval
OR	odds-ratio
HR	hazard ratio
FPR	fibrosis progression rate
SMU	standardized Metabir units
LD	linkage disequilibrium

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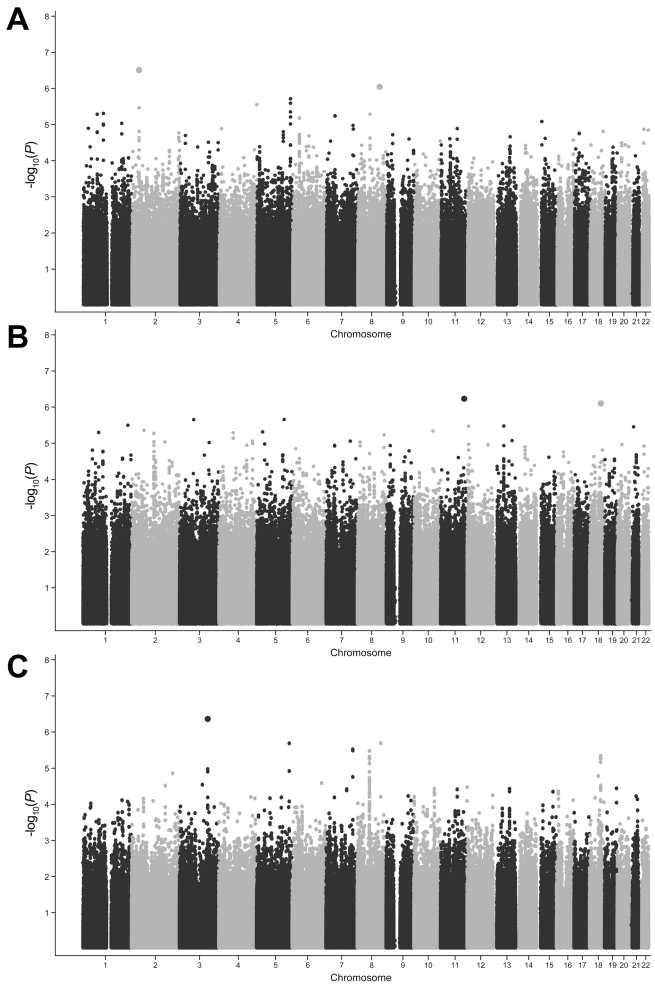

Figure 1.

Manhattan plots of genome-wide analyses of liver fibrosis using (**A**) the *binary F0-1/F3-4* phenotype (949 F0-1/F3-4 patients), (**B**) the *duration F0-1/F3-4* phenotype (872 F0-1/F3-4 patients with available duration of infection) and (**C**) the *QTF* phenotype (1,064 patients with available duration of infection). Larger points correspond to SNPs producing a *P*-value<10^{-6}. All the analyses were performed on 780,650 genotyped or imputed SNPs (Patients & Methods).

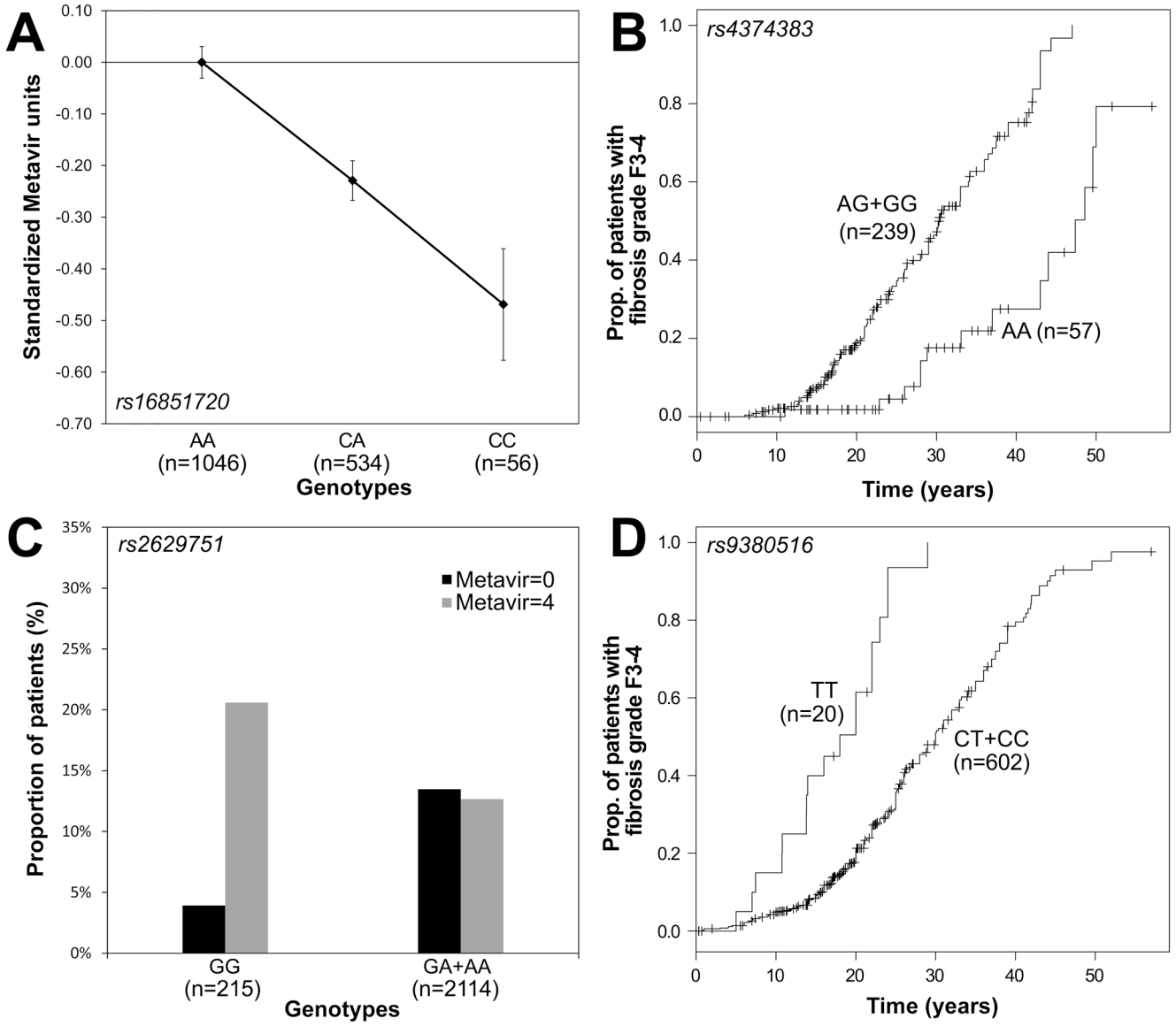

Figure 2.

Genotype/phenotype relationships for the four SNPs most associated with liver fibrosis in the final combined cohort. (**A**) Standardized Metavir units (SMU; Patients & Methods) for the three genotypes of SNP rs16851720 (located within *RNF7*), identified using the *QTF* phenotype. Bars represent standard errors (SEM) of each mean SMU. Genotype AA was used as the reference (SMU=0). (**B**) Survival curves for SNP rs4374383 (located within *MERTK*) identified using the *duration F0-1/F3-4* phenotype, in the subsample of transfused patients. (**C**) Proportions of all HCV-infected patients with Metavir scores of F0 and F4, by genotype at rs2629751 (located within *GLT8D2*), a replicated SNP identified using the *binary F0/F4* phenotype. (**D**) Survival curves for SNP rs9380516 (located near *TULP1*), a replicated SNP identified using the *duration F0-1/F3-4* phenotype stratified in male patients.

Table 1

Clinical and demographic characteristics of the two primary cohorts.

Covariate	Category	French cohort[a]	Swiss cohort[a]	Total
Sex				
	male	209 (44.8%)	433 (62.4%)	642 (55.3%)
	female	258 (55.2%)	261 (37.6%)	519 (44.7%)
Metavir score				
	F0	41 (8.8%)	77 (11.1%)	118 (10.2%)
	F1	244 (52.2%)	217 (31.3%)	461 (39.7%)
	F2	20 (4.3%)	192 (27.7%)	212 (18.3%)
	F3	93 (19.9%)	86 (12.4%)	179 (15.4%)
	F4	69 (14.8%)	122 (17.6%)	191 (16.5%)
Alcohol consumption				
	Low (<40g/day)	399 (85.4%)	542 (78.1%)	941 (81.1%)
	High[b] (>40g/day)	68 (14.6%)	132 (19.0%)	200 (17.2%)
	Not available (NA)	0 (0.0%)	20 (2.9%)	20 (1.7%)
HCV mode of acquisition				
	IDU[c]	157 (33.6%)	290 (41.8%)	447 (38.5%)
	Blood transfusion	205 (43.9%)	133 (19.2%)	338 (29.1%)
	Others[c]/NA	105 (22.5%)	271 (39.0%)	376 (32.4%)
HCV genotype				
	1	294 (63.0%)	362 (52.2%)	656 (56.5%)
	2	41 (8.8%)	67 (9.7%)	108 (9.3%)
	3	75 (16.1%)	193 (27.8%)	268 (23.1%)
	4	10 (2.1%)	57 (8.2%)	67 (5.8%)
	NA	47 (10.1%)	15 (2.2%)	62 (5.3%)
Age at infection				
	Patients with available data	450 (96.4%)	614 (88.5%)	1064 (91.6%)

Covariate	Category	French cohort[a]	Swiss cohort[a]	Total
	Age at infection < 20 years	139 (29.8%)	304 (43.8%)	443 (38.2%)
	Age at infection ≥20 years	311 (66.6%)	310 (44.7%)	621 (53.5%)
	Mean (SD) age at infection (*years*)	28.00 (12.93)	21.20 (10.66)	24.08 (12.14)
	Mean (SD) duration of infection (*years*)	20.17 (9.25)	22.39 (10.61)	21.45 (10.11)
	Mean (SD) FPR (*Metavir units/year*)	0.11 (0.16)	0.11 (0.15)	0.11 (0.16)
Total		467	694	1,161

[a]French patients were included if they were not co-infected with HIV or HBV and had no coexisting chronic liver disease. We also preferentially enrolled patients if (i) they had F0-1 or F3-4 Metavir scores, (ii) their presumed date of HCV acquisition was known and (iii) they had low levels of alcohol consumption. Swiss patients with known HIV or active HBV co-infection were excluded for the purposes of this study. For both cohorts, relevant factors were retrieved from clinical databases, including sex, age at infection, mode of HCV acquisition and HCV genotype.

[b]This category includes past heavy drinkers.

[c]IDU stands for injecting drug use. Other modes of HCV acquisition include situations at risk of exposure to blood (e.g. health workers) and invasive protocols (e.g. surgery, tattoo, piercing).

Table 2

Replicated SNPs from our GWA analyses of different phenotypes of liver fibrosis in patients with chronic hepatitis C infection.

SNP	Chr	Closest gene	Distance	m/M[a]	mAF	Phenotype	Model[a]	Discovery *P*-value[b]	Initial combined *P*-value[b]	Total combined *P*-value[b]	Hetero-geneity *P*-value[c]	Genotypes (n)	Effect[d]
rs16851720	3	*RNF7*	Intronic	C/A	0.19	*QTF*	Additive	4.5×10^{-7}	6.2×10^{-8}	8.9×10^{-9}	0.37	AA (1046) AC (534) CC (56)	0 −0.23 (−0.31 - −0.15) −0.46 (−0.54 - −0.38)
rs4374383	2	*MERTK*	Intronic	A/G	0.42	*Duration F0-1/F3-4* in transfused patients	Recessive	2.7×10^{-8}	2.1×10^{-9}	1.1×10^{-9}	0.81	AG/GG (257) AA (62)	1 0.19 (0.10–0.37)
rs9380516	6	*TULP1*	21	T/C	0.17	*Duration F0-1/F3-4* in male patients	Recessive	4.0×10^{-6}	9.6×10^{-7}	5.4×10^{-7}	0.88	TC/CC (602) TT (20)	1 4.53 (2.78–7.39)
rs2629751	12	*GLT8D2*	Intronic	G/A	0.31	*Binary F0/F4*	Recessive	1.6×10^{-5}	1.4×10^{-7}	1.4×10^{-7}	0.37	GA/AA (546) GG (49)	1 7.10 (2.94–20.74)
rs883924	9	*LOC340515*	43	A/G	0.26	*Duration F0-1/F3-4* age at infection > 20y	Recessive	3.7×10^{-6}	4.6×10^{-7}	1.8×10^{-6}	0.15	AG/GG (765) AA (68)	1 2.69 (1.88–3.85)
rs7800244	7	*PKD1L1*	34	T/G	0.18	*Binary F0-1/F3-4*	Dominant	1.8×10^{-6}	2.9×10^{-7}	3.4×10^{-6}	0.06	GG (1210) TG/TT (602)	1 0.61 (0.49–0.75)
rs6485480	11	*ALKBH3*	18	T/C	0.22	*Binary F0/F4*	Additive	2.5×10^{-5}	5.1×10^{-7}	3.2×10^{-5}	0.01	CC (359) TC (201) TT (36)	1 1.86 (1.41–2.46) 3.46 (1.99–6.05)

[a] The first allele m refers to the minor allele, and M to the major allele, in the combined cohort. The genetic model is defined on the basis of the minor allele.

[b] The discovery *P*-value was obtained in the primary cohort of 1,161 Swiss and French patients; the initial combined *P*-value was obtained in the cohort of 2,123 patients, including Swiss and French primary cohorts (Table 1), and our replication cohort (Cornell, Marseilles and International cohorts; Supplementary Table 1); the total combined *P*-value was obtained by combining this cohort of 2,123 patients with the sample of 219 Australian patients (Supplementary Table 1) previously genotyped in another GWA study.[19]

[c] Heterogeneity was tested among primary, replication and Australian cohorts using the Cochran's Q-statistics.[46]

[d] The effect is computed from the total sample of 2,342 patients. Effects correspond to the standardized difference in QTF rates, relative to the first genotype (Patients & Methods), when using the quantitative *QTF* phenotype, hazard ratios when the *duration* phenotype is considered, and odds-ratios when the *binary* phenotype is considered.