Mx1, OAS1 and OAS2 polymorphisms are associated with the severity of liver disease in HIV/HCV-coinfected patients: A cross-sectional study

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The mechanisms involved in the chronic hepatitis C progression are incompletely understood. The aim was to analyze the association between 2′,5′-oligoadenylate synthetase 1,2 and 3 (OAS1-3) and myxovirus resistance proteins 1 (Mx1) polymorphisms and severity of liver disease in human immunodeficiency virus (HIV)/hepatitis C virus (HCV) coinfected patients. We performed a cross-sectional study in 219 patients that underwent a liver biopsy. DNA genotyping for Mx1 (rs469390), OAS1 (rs2285934), OAS2 (rs1293762) and OAS3 (rs2010604) was performed by using GoldenGate assay. The outcome variables in liver biopsy were: (i) significant fibrosis (F ≥ 2); (ii) moderate activity grade (A ≥ 2). Additive model of inheritance for genetic association test was used. The likelihood of having significant fibrosis (F ≥ 2) was lower in patients carrying OAS2 rs1293762 A allele [adjusted odds ratio (aOR) = 0.51; p = 0.040]. Besides, the likelihood of having moderate activity grade (A ≥ 2) was higher in patients carrying Mx1 rs464397 C allele (aOR = 1.63; p = 0.028) and Mx1 rs469390 G allele (aOR = 1.97; p = 0.005), while it was lower in patients carrying OAS1 rs2285934 A allele (aOR = 0.64; p = 0.039) and OAS2 rs1293762 A allele (aOR = 0.41; p = 0.009). In conclusion, Mx1 and OAS1-2 polymorphisms were associated with the severity of liver disease in HIV/HCV-coinfected patients, suggesting a significant role in the progression of hepatic fibrosis.

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of viral infections such as tick-borne encephalitis, West Nile virus, dengue virus, coronavirus, and hepatitis B. Regarding to HCV infection, MX1 rs2071430 polymorphism has been related to HCV clearance during acute HCV infection and after interferon (IFN) therapy, and higher severity of liver disease in HCV-monoinfected patients. However, to our knowledge, there is no report related to OAS/MX SNPs and severity of liver disease in HIV/HCV coinfected patients.

The aim of this study was to analyze the association between MX1 and OAS1-3 polymorphisms and severity of liver disease in HIV/HCV coinfected patients.

Results

Characteristics of the study population. Table 1 shows the epidemiological and clinical characteristics of 219 HIV/HCV-coinfected patients at the time of liver biopsy. Regarding liver biopsy, 49.8% had significant fibrosis (F ≥ 2), 52.6% had moderate necroinflammatory activity (A ≥ 2), and 15.3% had alanine aminotransferase (ALT) ≥ 150 IU/L.

MX1 and OAS1-3 polymorphisms. Table 2 shows the characteristics of SNPs, which displayed missing values < 5% and were in HWE (p > 0.05). When SNPs frequencies in HIV/HCV coinfected patients were compared to the SNPs frequencies in healthy subjects from the IBS, we only found differences between groups for MX1 rs469390 AA (p = 0.011) and MX1 rs469390 AG (p = 0.036) genotypes.

Association between MX1 and OAS1-3 polymorphisms and liver disease. Table 3 shows the relationship of MX1 and OAS1-3 polymorphisms with severity of liver disease under a model of additive inheritance, which was the genetic model that best fitted our data. The trend of having significant fibrosis (F ≥ 2) was lower in patients carrying OAS2 rs1293762 A allele (p = 0.023). The trend of having moderate activity grade (A ≥ 2) was higher in patients carrying MX1 rs464397 C allele (p = 0.015) and MX1 rs469390 G allele (p = 0.003); while it was lower in patients carrying OAS1 rs2285934 A allele (p = 0.026), OAS2 rs1293762 A allele (p = 0.002), and it was nearly significant for OAS3 rs2010604 C allele (p = 0.095). Moreover, the likelihood of having significant fibrosis (F ≥ 2) in liver biopsy was lower in patients carrying OAS2 rs1293762 A allele [adjusted odds ratio (aOR) = 0.51; p = 0.040]. Besides, the likelihood of having moderate activity grade (A ≥ 2) was higher in patients carrying MX1 rs464397 C allele (aOR = 1.63; p = 0.028) and MX1 rs469390 G allele (aOR = 1.97; p = 0.005), while it was lower in patients carrying OAS1 rs2285934 A allele (aOR = 0.64; p = 0.039) and OAS2 rs1293762 A allele (aOR = 0.41; p = 0.009).

Figure 1 shows the relation between MX1 and OAS1-3 genetic polymorphisms and serum ALT. We found that patients carrying MX1 rs464397 CC genotype and MX1 rs469390 GG genotype had higher values of serum ALT and percentage of patients with ALT ≥ 150 IU/L. However, we did not find any significant association in multivariate regression analysis (data not shown). Moreover, we also analyzed the relation between MX1 and OAS1-3 polymorphisms and serum aspartate aminotransferase (AST), but we did not find any association (data not shown).

Table 4 shows the relationship among haplotype of MX1 gene (rs464397, rs458582, rs469390) and OAS1-3 cluster (rs2285934, rs2285933, rs2010604, rs739903, rs1293762) and severity of liver disease. The likelihood of having moderate activity grade (A ≥ 2) in liver biopsy was higher in patients with MX1 CTG haplotype (aOR = 1.44; p < 0.001) and lower in patients with MX1 CTA haplotype (aOR = 0.62; p = 0.007). Moreover, the likelihood of having moderate activity grade (A ≥ 2) in liver biopsy was lower in patients with OAS1-3 AGCTA haplotype (aOR = 0.78; p = 0.008).

Discussion

To our knowledge, this study is the first description of the relationship between MX1 and OAS1-3 polymorphisms and severity of liver disease in HIV/HCV-coinfected patients. The major findings of the present study were that MX1 rs464397 C allele, MX1 rs469390 G allele, and MX1 CTG haplotype were associated with higher odds of having moderate activity grade (A ≥ 2) in liver biopsy, while OAS1 rs2285934 A allele, OAS2 rs1293762 A allele, and OAS1-3 AGCTA haplotype were related to lower likelihood of having moderate activity grade (A ≥ 2). Besides, OAS2 rs1293762 A allele was related to lower likelihood of having significant fibrosis (F ≥ 2) in liver biopsy. Thus, MX1 and OAS1-2 polymorphisms might play a role in developing of liver disease in HIV/HCV-coinfected patients.

In our study, we included a control group to show that our study cohort had allelic and genotypic frequencies similar to the general population. However, we found significant differences in allelic and genotypic frequencies of MX1 rs469390 between HIV/HCV coinfected patients and Iberian populations in Spain from HapMap data. This observation might suggest that MX1 rs469390 could be associated to innate resistance to HCV infection. However, our study design is not adequate to draw such conclusions due to a number of factors that we do not control. For example, our cohort may have a survival bias because patients were more than 20 years of median with HIV and HCV infections. In addition, patients were selected where they were candidates for antiviral treatment against HCV because progression of liver disease was suspected.

In the setting of CHC, the immune system initially attempts to eradicate the HCV, but besides, probably promotes hepatocyte damage and fibrosis through direct cellular toxicity and the release of inflammatory cytokines. The innate immune response against viruses produce type I IFN (alpha, beta), which induces the expression of MX1 protein. Although its effect against HCV is not well documented, high levels of Mx1 protein have been detected in IFN-treated HCV patients with sustained virological response. In fact, MX1 rs2071430 polymorphism, in the promoter region, has been related to MX1 gene expression, spontaneous viral clearance during acute HCV infection, higher hepatic fibrosis score and hepatitis activity grade, and response to IFN therapy in HCV-monoinfected patients. In the in silico analysis performed, we found that MX1 rs464397 seems to be implicated in both proximal (open chromatin region, transcription factor binding sites and histone
### Table 1. Clinical and epidemiological characteristics of HIV/HCV-coinfected patients.

Values are expressed as absolute numbers (%) and median (percentile 25; percentile 75). Sometimes, the percentages were not calculated from all patients because some data was missing. Abbreviations: BMI, body mass index; HOMA, homeostatic model assessment; HCV, Hepatitis C virus; CDC category C, centers for disease control and prevention classification system, clinical category C which includes any condition listed in the CDC’s 1987 surveillance case definition of AIDS; PI-based, protein inhibitor-based therapy; NNRTI-based, non-nucleoside

| Characteristics                        | All Patients |
|----------------------------------------|--------------|
| No.                                    | 219          |
| Male                                   | 162 (74%)    |
| Age, years                             | 39.8 (37.4; 44) |
| Epidemiological history                |              |
| HIV acquired by IVDU                   | 193 (88.1%)  |
| Years since HCV infection              | 21.3 (17; 24.3) |
| High alcohol intake                    | 119 (54.3%)  |
| High alcohol intake at biopsy study    | 30 (13.7%)   |
| CDC category C                         | 60 (27.4%)   |
| Metabolic markers                      |              |
| BMI, kg/m²                              | 22.3 (20.8; 24.6) |
| BMI ≥ 25 kg/m²                         | 50 (23%)     |
| HOMA                                    | 2.10 (1.27; 3.75) |
| HOMA ≥ 3                               | 71 (33.5%)   |
| Antiretroviral therapy                 |              |
| cART                                   | 183 (83.6%)  |
| Time on cART, years                    | 4.4 (2.5; 6.6) |
| Current cART protocols                 |              |
| Non-treated                            | 36 (16.4%)   |
| PI-based                               | 50 (22.8%)   |
| NNRTI-based                            | 114 (52.1%)  |
| NRTI-based                             | 19 (8.7%)    |
| HIV markers                            |              |
| Nadir CD4+, T cells/μL                 | 189 (83; 315) |
| CD4+, T cells/μL                       | 467 (324; 672) |
| HIV-RNA < 50 copies/mL                 | 162 (74%)    |
| HCV markers                            |              |
| HCV genotype (n = 215)                 |              |
| 1                                      | 122 (56.7%)  |
| 2                                      | 5 (2.3%)     |
| 3                                      | 50 (23.3%)   |
| 4                                      | 38 (17.7%)   |
| HCV RNA > 500,000 UI/mL (n = 216)      | 161 (74.5%)  |
| Liver biopsy (Metavir score)           |              |
| Hepatic fibrosis                       |              |
| F0                                     | 25 (11.4%)   |
| F1                                     | 85 (38.8%)   |
| F2                                     | 60 (27.4%)   |
| F3                                     | 26 (11.9%)   |
| F4                                     | 23 (10.3%)   |
| Activity grade (n = 215)               |              |
| A ≤ 1                                  | 102 (47.5%)  |
| A2                                     | 89 (41.4%)   |
| A3                                     | 24 (11.2%)   |
| Aminotransferases                      |              |
| ALT (IU/L) (n = 219)                   | 72 (45; 114) |
| ALT ≥ 150 IU/L                         | 33 (15.3%)   |
| AST (IU/L) (n = 215)                   | 55 (37; 82)  |
| AST ≥ 100 IU/L                         | 33 (15.3%)   |
| AST: ALT (n = 215)                     | 0.79 (0.63; 0.97) |
| AST: ALT < 1                           | 110 (50.2%)  |
reverse transcriptase inhibitor-based therapy; 3NRTI-based, triple nucleoside regimen; HIV: Human immunodeficiency virus; HIV-RNA, HIV plasma viral load; HCV-RNA, HCV plasma viral load; ALT, alanine aminotransferase; AST Aspartate Aminotransferase.

markers) and distal regulation. Furthermore, rs464397 seems also be involved in RNA binding protein-mediated post-transcriptional regulation as well as to be a SNP with experimental expression quantitative trait loci (eQTL) evidence. On the other hand, Mx1 rs469390 polymorphism is a missense variant (V379I) that might not affect protein structure; but it seems influence an exonic splicing enhancer motif and it might be involved in protein domain abolishing. In this way, Mx1 rs469390 polymorphism could decrease expression of MX1, getting worse the control of HCV, and might therefore be related to the increase of sustained inflammation and progression of liver disease.

The OAS proteins play an important role in the innate immune response and genetic variants in the OAS genes are known to affect OAS activity and are associated with outcome of viral infections\(^5\). The OAS1 rs2660, rs10774671, and rs3741981 polymorphisms have been related to persistent HCV infection\(^11,17\) and progression of HCV disease\(^18\); and OAS1 polymorphism on exon 7 splice acceptor site with response to IFN therapy in HCV infected patients\(^19\). The OAS2 polymorphism rs1293762 has been associated with predisposition to CHC\(^20\). In our study, OAS1-3 polymorphisms were related to lower likelihood of having significant liver disease (A ≥ 2 and/or F ≥ 2), indicating that the A-allele conferred a protective effect with respect to liver disease progression. OAS1 rs2285934 and OAS2 rs1293762 polymorphisms are located in intron 3 and intron 2, respectively. In the in silico analysis, both SNPs seem to be involved in RNA binding protein-mediated post-transcriptional gene regulation. In this way, OAS1 rs2285934 and OAS2 rs1293762 polymorphisms could increase expression of OAS1 and OAS2, improving the control of HCV, and might therefore be related to the decrease of sustained inflammation and progression of liver disease.

As discussed above, the presence of Mx1 and OAS1-3 polymorphisms may be related to have moderate or significant liver disease. This association that we observed might be due to the proximity to other SNPs that may modulate Mx1 gene expression, or a direct effect of Mx1 and OAS1-3 polymorphisms analyzed in this study. On the one hand, haplotype analysis showed a stronger association with liver disease than that found with each polymorphism alone. On the other hand, the in silico analysis showed that polymorphisms alone could have some influence on the expression of the proteins involved. However, it would be necessary to demonstrate if these Mx1 and OAS1-3 polymorphisms may modify the expression of Mx1 and OAS1-3 genes.

The impact of OAS/MX1 SNPs on fibrosis progression in CHC has not been picked up in the GWAS previously carried out\(^21,22\). However, we found a relatively strong association between OAS/MX1 SNPs and the severity of liver disease in our HIV/HCV coinfected patients. This could be because: (a) GWAS usually does not have the power to detect all, only the biggest effects. Besides, note that strong statistical adjustments on the p-value are carried out for correcting multiple testing; (b) we studied a SNP candidate with MAF greater than 20%. In these cases, an individual effect is more easily detected when the size of sample is small\(^23\); (c) we should not discard the effect of HCV infection on the immune system, which could show the influence of OAS/MX1 polymorphisms on CHC progression.

Finally, several aspects have to be taken into account for the correct interpretation of the results. Firstly, this is a cross-sectional study with a limited number of patients, which could limit achieving statistically significant values. Besides, the cross-sectional design nature of the analyses may be a confounding factor. Second, data were collected retrospectively, which entails a lack of uniformity. Thirdly, all selected patients met a set of criteria for starting HCV treatment (e.g., no alcohol abuse, high CD4 cell counts, controlled HIV replication, and good treatment adherence), and this may have introduced a selection bias. Fourthly, regarding the statistical significance, there is a considerable controversy about adjusting the "p-value" after multiple tests on clinical-oriented studies\(^24,25\). In our study, there was a hypothesis supported by theory and previous reports that related Mx1 and OAS1-3 polymorphisms to severity and/or outcome of viral infections\(^5-9,16\), including HCV infection\(^10-12\). Therefore, we were not literally doing a random search of a meaningful result, and our results should not be affected by the fact of carrying out a high number of statistical tests. Fifthly, this study was performed on patients with European ancestry, and it would be interesting to perform these analyses on different ethnic groups. Finally, our study only included HIV/HCV-coinfected patients and it would be interesting to know the role of studied polymorphisms in HCV monoinfected patients, but we did not have access to a cohort of HCV monoinfected patients.

In conclusion, Mx1 and OAS1-2 polymorphisms were associated with the severity of liver disease in patients coinfected with HIV and HCV, suggesting that these polymorphisms might play a significant role in the progression of hepatic fibrosis. Further analysis involving large numbers of patients are needed to corroborate these associations that we have found.

Materials and Methods

Study population. We performed a cross-sectional study in 219 HIV/HCV-coinfected patients that underwent a liver biopsy between September 2000 and November 2008. The study was conducted in accordance with the Declaration of Helsinki and patients gave their written consent for the study. The Institutional Review Board and the Research Ethic Committee of the Instituto de Salud Carlos III approved the study. Each participating patient signed an informed consent form.

Liver biopsies were performed on patients who were potential candidates for anti-HCV therapy and had not received previous IFN therapy (naïve for HCV-treatment). Selection criteria were: no clinical evidence of hepatic decompensation, detectable HCV RNA by polymerase chain reaction (PCR), negative hepatitis B surface antigen, availability of DNA sample, CD4+ lymphocyte count higher than 200 cells/μL, and stable combination antiretroviral therapy (cART) for at least 6 months before study entry or no need for cART according to treatment...
guidelines used in the study period\(^2\)\(^6\)\(^7\). Patients with active opportunistic infections, active drug addiction, and other concomitant severe diseases were excluded. All subjects included in our study were European white.

**Epidemiological and clinical data.** Clinical and epidemiological data were obtained from medical records. The duration of HCV infection for patients with a history of intravenous drug use was estimated starting from the first year they shared needles and other injection paraphernalia, which are the most relevant risk factors.

| SNPs | HWE | HIV/HCV group (n = 219) | IBS group (n = 107) | \(\chi^2\) test (a) | \(\chi^2\) test (b) |
|------|------|-------------------------|---------------------|-------------------|-------------------|
| **Mx1 rs464397** | 0.420 | C 53% CC 26% | C 53% CC 28% | 0.906 | 0.802 |
| | | T 47% CT 53% | T 47% CT 50% | | |
| | | TT 21% | TT 22% | | |
| **Mx1 rs469390** | 0.410 | A 56% AA 30% | A 64% AA 45% | 0.208 | 0.011 |
| | | G 44% AG 52% | G 35% AG 39% | | |
| | | — — GG 17% | — — GG 16% | | |
| **OAS1 rs2285934** | 0.450 | C 66% CC 45% | C 67% CC 41% | 0.956 | 0.572 |
| | | A 34% CA 42% | A 33% CA 51% | | |
| | | — — AA 13% | — — AA 8% | | |
| **OAS2 rs1293762** | 0.790 | C 54% CC 29% | C 52% CC 25% | 0.824 | 0.531 |
| | | A 46% CA 49% | A 48% CA 54% | | |
| | | — — AA 22% | — — AA 21% | | |
| **OAS3 rs2010604** | 0.360 | G 67% GG 17% | G 66% GG 42% | 0.955 | 0.573 |
| | | C 33% GC 41% | C 33% GC 49% | | |
| | | — — CC 12% | — — CC 8% | | |

Table 2. Summary of Hardy Weinberg Equilibrium and frequencies of alleles and genotypes for **Mx1** and **OAS1-3** polymorphisms in HIV/HCV coinfected patients compared to Iberian populations in Spain from HapMap data (http://www.ncbi.nlm.nih.gov/bioproject/59819). Statistically significant differences are shown in bold. P-values were calculated by Chi-squared test; (a), differences between allele frequencies; (b), differences between genotype frequencies. Abbreviations: HWE, Hardy Weinberg Equilibrium; HIV, human immunodeficiency virus; HCV, hepatitis C virus; IBS, Iberian populations in Spain; OAS, 2′5′ oligoadenylate synthetase; Mx1, myxovirus resistance proteins.

| SNPs | Unadjusted | Adjusted |
|------|------------|----------|
| **Mx1 rs464397** | TT | CT | CC | P\(^{a(b)}\) | aOR (95%CI) | p\(^{b(b)}\) |
| F ≥ 2 | 42.2% (19/45) | 50.0% (58/116) | 55.2% (32/58) | 0.198 | 1.15 (0.75; 1.77) | 0.501 |
| A ≥ 2 | 38.6% (17/44) | 52.6% (60/114) | 63.2% (36/57) | 0.015 | 1.63 (1.05; 2.52) | 0.028 |
| **Mx1 rs469390** | AA | AG | GG | P\(^{a(b)}\) | aOR (95%CI) | p\(^{b(b)}\) |
| F ≥ 2 | 39.4% (26/66) | 53.5% (61/114) | 55.3% (21/38) | 0.077 | 1.22 (0.80; 1.88) | 0.347 |
| A ≥ 2 | 40% (25/65) | 53.6% (60/112) | 70.3% (26/37) | 0.003 | 1.97 (1.23; 3.16) | 0.005 |
| **OAS1 rs2285934** | CC | AC | AA | P\(^{a(b)}\) | aOR (95%CI) | p\(^{b(b)}\) |
| F ≥ 2 | 52.0% (51/98) | 49.5% (46/93) | 42.9% (12/28) | 0.415 | 0.88 (0.58; 1.33) | 0.539 |
| A ≥ 2 | 60.4% (58/96) | 48.4% (44/91) | 39.3% (11/28) | 0.026 | 0.64 (0.42; 0.98) | 0.039 |
| **OAS2 rs1293762** | CC | AC | AA | P\(^{a(b)}\) | aOR (95%CI) | p\(^{b(b)}\) |
| F ≥ 2 | 60.9% (39/64) | 47.7% (51/107) | 39.6% (19/48) | 0.023 | 0.51 (0.26; 0.97) | 0.040 |
| A ≥ 2 | 66.7% (42/63) | 51.0% (53/104) | 37.5% (18/48) | 0.002 | 0.41 (0.21; 0.80) | 0.009 |
| **OAS3 rs2010604** | GG | CG | CC | P\(^{a(b)}\) | aOR (95%CI) | p\(^{b(b)}\) |
| F ≥ 2 | 52.5% (53/101) | 48.9% (44/90) | 44.4% (12/27) | 0.431 | 0.91 (0.59; 1.38) | 0.651 |
| A ≥ 2 | 59% (59/100) | 48.3% (42/87) | 44.4% (12/27) | 0.095 | 0.74 (0.48; 1.13) | 0.169 |

Table 3. Summary of the differences of genotypic frequencies (\(Mx1 rs464397, MX1 rs469390, OAS1 rs2285934, OAS2 rs1293762 and OAS3 rs2010604\)) in relation to severity of liver disease and the adjusted logistic regression analysis for the polymorphisms in HIV/HCV coinfected patients. Statistically significant differences are shown in bold. \(^{(a)}\)P-values were calculated by Chi-squared test for trend (linear by linear association); \(^{(b)}\)P-values were calculated by multivariate logistic regression adjusted by the most important clinical and epidemiological characteristics (see statistical analysis section). Sometimes, the percentages were not calculated from all patients because some data was missing. Abbreviations: 95%CI, 95% of confidence interval; aOR, adjusted odds ratio; p-value, level of significance; A ≥ 2, moderate activity (Metavir); F ≥ 2, significant fibrosis (Metavir); OAS, 2′5′ oligoadenylate synthetase; Mx1, myxovirus resistance proteins.
Figure 1. Summary of the differences of serum ALT according to genotypes of Mx1 rs464397, MX1 rs469390, OAS1 rs2285934, OAS2 rs1293762 and OAS3 rs2010604 in HIV/HCV coinfected patients. P-values were calculated by Student’s t-test and Chi-squared test. Abbreviations: OAS, 2′5′oligoadenylate synthetase; Mx1, myxovirus resistance proteins.
Diego, California, USA). www.cegen.org/) for DNA genotyping by using GoldenGate assay with VeraCode Technology (Illumina Inc., San 

Qiagen, Hilden, Germany). DNA samples were sent at the Spanish National Genotyping Center (CeGen; http://

linkage disequilibrium (LD) (Fig. 2).

and

OAS

according to linkage disequilibrium (LD) (Fig. 2): two SNPs at

in exon 6 (missense), and

rs2010604 in 3

significant associations when working with small sample sizes34. With this setting, we found three SNPs in

(ii) minor allelic frequency (MAF) greater than 20% in European people in order to have guarantees to obtain

ria were: (i) SNPs located in putative regulatory regions, or splicing control elements (SCE), or missense variants;

cgi-perl/gbrowse/hapmap_B35/) and NCBI (dbSNP) (http://www.ncbi.nlm.nih.gov/entrez/). The selection crite-

OAS1-3

genes (chromosome 12) were selected using the databases of HapMap Project (http://snp.cshl.org/

grade) was scored as follows: A0, no activity; A1, mild activity; A2, moderate activity; A3, severe activity.

F ≥ 2

A ≥ 2

Freq. aOR (95% CI) p aOR (95% CI) p

C T G

0.377 1.44 (0.92–2.26) 0.109 2.38 (1.45–3.91) <0.001

T G A

0.413 0.85 (0.54–1.34) 0.487 0.68 (0.42–1.09) 0.104

C T A

0.141 0.62 (0.32–1.22) 0.165 0.39 (0.19–0.79) 0.007

rs2285934

rs2010604

rs739903

rs1293762

A G C T A

0.281 0.78 (0.50–1.21) 0.261 0.55 (0.35–0.87) 0.008

C G G T A

0.133 0.66 (0.34–1.35) 0.195 0.93 (0.50–1.76) 0.836

C C G T C

0.239 1.14 (0.70–1.84) 0.592 1.40 (0.85–2.29) 0.181

C G G T C

0.250 1.33 (0.82–2.16) 0.250 1.45 (0.88–2.39) 0.140

Table 4. Association of haplotype of Mx1 gene and OAS1-3 cluster with severity of liver disease in HIV/

HCV coinfected patients. Statistically significant differences are shown in bold. P-values were calculated by

multivariate logistic regression adjusted by the most important clinical and epidemiological characteristics (see statistical analysis section). Haplotypes with frequencies less than 5% have not included. Abbreviations: aOR, adjusted odds ratio; 95%CI, 95% of confidence interval; p-value, level of significance; A ≥ 2, moderate activity (Metavir); F ≥ 2, significant fibrosis (Metavir); OAS, 2′5′oligoadenylate synthetase; Mx1, myxovirus resistance proteins.

practices for HCV transmission28. The duration of HCV infection was not calculated when the date of initiation of their HCV infection could not be determined with certainty. Consumption of more than 50 g of alcohol per day for at least 12 months was considered as a high intake. Body mass index (BMI) was calculated as the weight in kilograms divided by the square of the height in meters.

Biochemistry panel was measured using an autoanalyzer Hitachi 912 (Boehringer Mannheim, Germany), while patients were fasting. The degree of insulin resistance was estimated for each patient using the homeostatic model assessment (HOMA)29: fasting plasma glucose (mmol/L) times fasting serum insulin (mU/L) divided by 22.5.

HCV assays. HCV infection was documented in all patients by enzyme-linked immunosorbent assay and PCR test. HCV genotype was determined by hybridization of biotin-labeled PCR products to oligonucleotide probes bound to nitrocellulose membrane strips (INNO-LiPA HCV II, Innogenetics, Ghent, Belgium). Plasma HCV-RNA viral load was measured by PCR (Cobas Amplicor HCV Monitor Test, Branchburg, NJ, USA) and real-time PCR (COBAS AmpliPrep/COBAS TaqMan HCV test); and results were reported in terms of international units per milliliter (IU/mL). The limit of detection varied between 10 IU/mL and 600 IU/mL depending on the HCV diagnostic test used at the time of biopsy. There was no patient with HCV viral loads below the limit of detection. The HCV RNA level of 500,000 UI/ml was chosen as cutoff for low vs. high viral load (circulating HCV RNA)30,31.

Liver biopsy. Liver biopsies were performed as we described previously32. The samples were always evaluated by the same pathologist, who was unaware of the patients’ clinical or laboratory data. Liver fibrosis and necroinflammatory activity were estimated according to Metavir score as follows33: F0, no fibrosis; F1, mild fibrosis; F2, significant fibrosis; F3, advanced fibrosis; and F4, definite cirrhosis. The degree of necroinflammation (activity grade) was scored as follows: A0, no activity; A1, mild activity; A2, moderate activity; A3, severe activity.

Genotyping of DNA polymorphisms. The most common SNPs in Mx1 gene (chromosome 21) and OAS1-3 genes (chromosome 12) were selected using the databases of HapMap Project (http://snp.cshl.org/cgi-perl/gbrowse/hapmap_B35/) and NCBI (dbSNP) (http://www.ncbi.nlm.nih.gov/entrez/). The selection criteria were: (i) SNPs located in putative regulatory regions, or splicing control elements (SCE), or missense variants; (ii) minor allelic frequency (MAF) greater than 20% in European people in order to have guarantees to obtain significant associations when working with small sample sizes34. With this setting, we found three SNPs in Mx1 gene (rs464397 in intron 3, rs458582 in intron 5, and rs469390 in exon 13 (missense)) and other five SNPs at OAS genes (OAS1 rs2285934 in intron 3, OAS2 rs1927962 in intron 2, OAS3 rs739903 in 3′UTR, OAS3 rs2285933 in exon 6 (missense), and OAS3 rs2010604 in 3′UTR). Finally, five polymorphisms were selected as tagSNPs according to linkage disequilibrium (LD) (Fig. 2): two SNPs at Mx1 (rs464397 and rs469390) and three SNPs at OAS (OAS1 rs2285934, OAS2 rs1927962, and OAS3 rs739903). Three SNPs (Mx1 rs458582, OAS3 rs2285933, and OAS3 rs2010604) were discarded for the analysis of individual association because these SNPs were at high linkage disequilibrium (LD) (Fig. 2).

Genomic DNA was extracted from peripheral blood with Qiagen kit (QIaAmp DNA Blood Midi/MaXi; Qiagen, Hilden, Germany). DNA samples were sent at the Spanish National Genotyping Center (CeGen; http://www.cegen.org/) for DNA genotyping by using GoldenGate assay with VeraCode Technology (Illumina Inc., San Diego, California, USA).
Moreover, for healthy subjects, the frequencies of alleles and genotypes for studied polymorphisms were obtained using the 1000 Genomes Project website (http://www.1000genomes.org/home), which provide a broad representation of common human genetic variation by applying whole-genome sequencing to a diverse set of individuals from multiple populations⁵. We select the IBS (Iberian populations in Spain) population that included 107 individuals.

Outcome variables. The primary outcome variables were related to severity of liver disease: significant fibrosis (F ≥ 2) and moderate activity grade (A ≥ 2). These outcomes were developed after a minimum follow-up time of 10 years with HCV infection.

Statistical analysis. Chi-square test, Chi-square test for trend (linear by linear association), Student’s t-test, and multivariate logistic regression analysis were used to investigate the relationship among Mx1 and OAS polymorphisms and severe liver disease. We included the SNP with the Enter algorithm (Forced Entry) and the covariables with the Stepwise algorithm (at each step, factors are considered for removal or entry: a p-value for entry and exit of 0.15 and 0.20, respectively). Thus, each logistic regression test was only adjusted by the most significant co-variables associated with each one of the outcome variables, avoiding the over-fitting of the regression. The covariables used were gender, age, alcohol intake at biopsy study, BMI, HOMA, nadir CD4+ T-cells, AIDS, undetectable HIV-RNA (< 50 copies/ml), CD4+ T-cells, time on cART, type of cART, HCV-RNA ≥ 500,000 IU/ml, time of HCV infection, and HCV genotype. Besides, we also used data of the PNPLA3 rs738409 polymorphism and the IL28B rs12980275 polymorphism, which have already been published by us and showed significant association with liver fibrosis in HIV/HCV coinfected patients⁶,⁷. The genetic association study was carried out according to the genetic model that best fit our data (additive, recessive, dominant, codominant, and overdominant). These analyses were tested according to the goodness of fit evaluated by Akaike information criterion (AIC) value and Bayesian information criterion (BIC). These analyses were performed by using the IBM SPSS Statistics for Windows, Version 21.0 (IBM Corp, Chicago, Armonk, NY, USA).

Hardy-Weinberg equilibrium (HWE) for all SNPs was assessed by a Chi-square test, considering equilibrium when p > 0.05. In addition, pair-wise linkage disequilibrium (LD) analysis was computed to detect the inter-marker relationship using the standardized $D'$ and $r^2$ values by Haploviev 4.2 software. Haplotype-based association testing was performed using Plink software (http://pngu.mgh.harvard.edu/~purcell/plink/) comparing each haplotype with the rest of haplotypes (there was no reference category). All p-values were two-tailed and statistical significance was defined as p < 0.05.
The *in silico* analysis for possible functional implication of each polymorphism was evaluated by using two web-tools: a) VarioWatch (http://genepipe.ncgm.sinica.edu.tw/variowatch/); b) rSNABase (http://rsnp.psych.ac.cn/).

References

1. Heim, M. H. & Thimme, R. Innate and adaptive immune responses in HCV infections. *Journal of hepatology* **61**, S14–S25, doi: 10.1016/j.jhep.2014.06.035 (2014).

2. Mengshol, J. A., Golden-Mason, L. & Rosen, H. R. Mechanisms of Disease: HCV-induced liver injury. *Nature clinical practice. Gastroenterology & hepatology* **4**, 622–634, doi: 10.1038/npgastp0961 (2007).

3. Lo Re, V. 3rd et al. Hepatic decompensation in antiretroviral-treated patients co-infected with HIV and Hepatitis C virus compared with hepatitis C virus-monoinfected patients: a cohort study. *Annals of internal medicine* **160**, 369–379, doi: 10.7326/m1-1829 (2014).

4. Schneider, W. M., Chevillotte, M. D. & Rice, C. M. Interferon-stimulated genes: a complex web of host defenses. *Genes Immun* **4**, 411–419, doi: 10.1038/sj.gene.6363984 (2003).

5. Hikijaka, M., Ohita, Y. & Mishiro, S. Identification of a Single Nucleotide Polymorphism in the MxA Gene Promoter (G/T at nt –88) Correlated with the Responsiveness of Patients to Interferon. *Interferon* **43**, 124–127 (2000).

6. Suzuki, F. et al. Single nucleotide polymorphism of the MxA promoter influences the response to interferon monotherapy in patients with hepatitis C viral infection. *Journal of viral hepatitis* **11**, 271–276, doi: 10.1111/j.1365-2893.2004.00509.x (2004).

7. Haller, O., Stadella, P., Schawmelle, M. & Kochs, G. MiGTPase: dynamin-like antiviral machines of innate immunity. *Trends in microbiology* **23**, 154–163, doi: 10.1016/j.tim.2014.12.003 (2015).

8. Fernandez, M. et al. In vivo and in vitro induction of MxA protein in peripheral blood mononuclear cells from patients chronically infected with hepatitis C virus. *The Journal of infectious diseases* **180**, 262–267, doi: 10.1086/314859 (1999).

9. Sadler, A. J. & Williams, B. R. G. Interferon-inducible antiviral effectors. *Nat Rev Immunol* **4**, 359–368 (2008).

10. Zhao, Y., Kang, H., Ji, Y. & Chen, X. Evaluate the relationship between polymorphisms of OAS1 gene and susceptibility to chronic hepatitis C with high resolution melting analysis. *Clinical and experimental immunology* **13**, 171–176, doi: 10.1046/j.1365-2123.2001.01934.x (2003).

11. Li, C.-Z. et al. Polymorphism of OAS-1 determines liver fibrosis progression in hepatitis C by reduced ability to inhibit viral replication. *Liver International* **29**, 1413–1421, doi: 10.1111/j.1478-3231.2009.02061.x (2009).

12. El Ashwari, M. K. et al. Single nucleotide polymorphism at exon 7 splice acceptor site of OAS1 gene determines response of hepatitis C virus patients to interferon therapy. *Journal of virology and hepatology* **26**, 843–850, doi: 10.1016/j.jvhepo.2014.10.005 (2015).

13. Barkhash, A. V., Kochneva, G. V., Chub, E. V., Mikhailova, S. V. & Romaschenko, A. G. Association between polymorphisms in OAS2 and CD209 genes and predisposition to chronic hepatitis C in Russian population. *Microbes and infection/Institut Pasteur* **16**, 445–449, doi: 10.1016/j.micinf.2014.02.004 (2014).

14. Rieger, S. et al. Impact of common risk factors of fibrosis progression in chronic hepatitis C. *Gut* doi: 10.1136/gutjnl-2014-306997 (2014).

15. Patin, E. et al. Genome-wide association study identifies variants associated with progression of liver fibrosis from HCV infection. *Gastroenterology* **143**, 1244–1252, e1241–e1212, doi: 10.1016/j.gastro.2012.07.097 (2012).

16. Park, J. H. et al. Distribution of allele frequencies and effect sizes and their interrelationships for common genetic susceptibility variants. *Nat Genet* **45**, 1413–1421, doi: 10.1038/nj.2013.198 (2013).

17. Perneger, T. V. What’s wrong with Bonferroni adjustments. *BMJ* **316**, 1236–1238 (1998).

18. Sterne, J. A. & Davey Smith, G. Sifting the evidence-what’s wrong with significance tests? *BMJ* **322**, 226–231 (2001).

19. Panel de expertos de Gesida Plan Nacional sobre el Sida y Asociación Española para el Estudio del Hígado. [Recommendations of Gesida/PNS/AEHH for the management and treatment of the adult patient co-infected with HIV and hepatitis A, B and C virus]. *Infectious Diseases* **31**, 61 e31–31, doi:10.1016/j.eimc.2009.12.001 (2010).

20. Ghany, M. G., Strader, D. B., Thomas, D. L. & Seeff, L. B. Diagnosis, management, and treatment of hepatitis C: an update. *Hepatology* **49**, 1335–1374, doi: 10.1002/hep.22759 (2009).

21. Thorpe, L. E. et al. Risk of hepatitis C virus infection among young adult injection drug users who share injection equipment. *American journal of epidemiology* **155**, 645–653 (2002).

22. Matthews, D. R. et al. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetesologia* **28**, 412–419 (1985).

23. Ascione, A. et al. Peginterferon alfa-2a plus ribavirin is more effective than peginterferon alfa-2b plus ribavirin for treating chronic hepatitis C virus infection. *Gastroenterology* **138**, 116–122, doi: 10.1053/j.gastro.2009.10.005 (2010).

24. Jimenez-Sousa, M. A. & H. 288A polymorphism is associated with early hepatitis C virus (HCV) treatment failure in human immunodefected virus (HIV)–HCV–coinfected patients. *J. Viral Hepat.* **16**, 358–368, doi: 10.1111/j.1365-2093.2009.00701.x (2013).

25. Resino, S. et al. An artificial neural network improves the non-invasive diagnosis of significant fibrosis in HIV/HCV coinfected patients. *The Journal of infection* **62**, 77–86, doi: 10.1016/j.jinf.2010.11.003 (2011).

26. Bedossa, P. & Poynard, T. An algorithm for the grading of activity in chronic hepatitis C. *The METAVIR Cooperative Study Group*. *Hepatology* (Baltimore, Md.) **24**, 289–293, doi: 10.1002/hep.510240201 (1996).

27. Houghton, E. P. & Park, J. W. Sample size and statistical power calculation in genetic association studies. *Genomics & informatics* **10**, 117–122, doi: 10.5808/GI.2012.10.2.117 (2012).

28. Ault, A. et al. A global reference for human genetic variation. *Nature* **526**, 68–74, doi: 10.1038/nature15393 (2015).
36. Jimenez-Sousa, M. A. et al. Impact of patatin-like phospholipase domain-containing 3 gene polymorphism (rs738409) on severity of liver disease in HIV/hepatitis C virus-coinfected patients. Aids 30, 465–470, doi: 10.1097/QAD.0000000000000908 (2016).
37. Guzman-Fulgencio, M. et al. IL28B polymorphisms are associated with severity of liver disease in human immunodeficiency virus (HIV) patients coinfected with hepatitis C virus. The Journal of infection 66, 170–178, doi: 10.1016/j.jinf.2012.10.025 (2013).

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Author Contributions
M.G.A. and S.R. performed all statistical analysis, interpretation of the data and wrote the manuscript. J.B. and S.R. participated in the study concept and design. J.B., T.A.E., C.D., and F.T., participated in patient selection, collection of samples and acquisition of data. D.P.T., M.A.J.S., and S.V.M. participated in sample preparation, DNA isolation and genotyping pre-procedure, and contributed with critical revision of the manuscript. S.R. supervised the study. All authors revised the manuscript from a draft by S.R.

Additional Information
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