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**Title page**

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**Title: *MBL2* genetic variants in HCV infection susceptibility, spontaneous viral clearance and peghylated-interferon plus ribavirin treatment response**

**Short title: *MBL2* polymorphisms and HCV infection**

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**Abstract**

Hepatitis C is disease that damages the liver and it is caused by the hepatitis C virus (HCV). The pathology became chronic in about 80% of the cases due to virus persistence in the host organism. The standard-of-care consists of peghylated-interferon plus ribavirin, however the treatment response is very variable and different host/viral factors may concur in the disease outcome.

The mannose-binding protein C (MBL) is a component of the innate immune system, able to recognize HCV and consecutively activating the immune response. MBL is encoded by *MBL2* gene and polymorphisms, two in the promoter region (H/L and X/Y) and three in exon 1 (at codon 52, 54 and 57) have been described as functionally influencing protein expression.

In this work 203 Italian HCV patients and 61 healthy controls were enrolled and genotyped for the five *MBL2* polymorphisms mentioned above in order to investigate their role in HCV infection susceptibility, spontaneous viral clearance and treatment response. *MBL2* polymorphisms were not associated with HCV infection susceptibility, and with spontaneous viral clearance, while *MBL2* O allele, O/O genotype, HYO haplotype and DP combined genotype (all correlated with low or deficient MBL expression) were associated with sustained virological response.

Moreover a meta-analysis to assess the role of *MBL2* polymorphisms in HCV infection susceptibility was also performed: YA haplotype could be associated with protection towards HCV infection.

**Keywords:** hepatitis C, innate immunity, *MBL2*

## Introduction

Hepatitis C is a common hepatic disease caused by infection with the Hepatitis C Virus (HCV) affecting globally around 130-150 million persons [1].

Patients are frequently asymptomatic during the acute phase of infection and most of them (about 80%) are not able to clear the virus: so the infection becomes chronic, possibly progressing to

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chronic liver disease, cirrhosis and hepatocellular carcinoma [2]. The HCV infected patients have been usually treated with pegylated interferon (PEG-IFN) plus ribavirin (RBV) [3] but this treatment accomplishes a sustained virological response (SVR) only in about 50% of the subjects [4] and it is frequently correlated with toxicity and with the generation of adverse effects [5].

The etiopathogenetic mechanism at the basis of liver injury as well as treatment response are not yet clearly understood and several efforts have been made to highlight the potential factors involved in this multifactorial phenotype [6, 7]. Individuals infected with HCV genotypes 1 [8] and 4 [9] are less responsive to PEG-IFN/RBV treatment than those with genotypes 2 and 3 [10].

Likewise it is widely assessed that the host immune system and particularly the innate components, representing the first line of host defence, could play an important role in the response to viral infection as well as to the treatment. Specifically toll-like receptor and RNA helicases retinoic acid inducible gene-I and melanoma differentiation antigen 5 have been reported as able to recognize virus and to activate the antiviral immune response [11], instead interferons are the principal cytokines produced, in particular the better known molecule associated with treatment response is Interferon-λ3, firstly identified by Ge et al. in 2009 [12]. Another important component of the innate immune system the mannose-binding protein C (MBL) potentially possesses a functional activity against HCV, since HCV E2 envelope glycoprotein has 11 N-linked glycosylation sites with mannose residues that could be recognized by this molecule [13]. The MBL binding leads to the activation of lectin pathway resulting in the complement cascade that finishes in the cleavage of C3 in C3a and C3b. C3b is deposited on virions or virus infected cells thus promoting virions aggregation, antigen presenting cells recruitment and other pro-inflammatory activities [14].

MBL serum level are known to be under genetic control: three point mutations in its encoding gene, *MBL2* (10q11.1-q21) at codon 52 (C>T, rs5030737), 54 (G>A, rs1800450) and 57 (G>A, rs1800451) in exon 1, named as D, B and C alleles, respectively (collectively designated as allele O,

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while the normal allele is called A) have been associated with low MBL concentrations [15]. In addition, other polymorphisms have been also described in the promoter region of the gene: the H and L at position 550 (rs11003125), are in linkage disequilibrium with the X and the Y variant at position 221 (rs7096206) that, combined with the variant at exon 1, form the six main haplotypes HYA, LYA, LXA, HYD, LYB and LYC, correlated with different protein serum level [16].

*MBL2* genetic variants, responsible for low MBL expression, have been previously associated with HCV infection susceptibility among Brazilians [17-19] and with less efficient HCV clearance during interferon therapy among Japaneses [20].

So, with the aim of better disclose the possible role of *MBL2* genetic variants in the context of HCV infection susceptibility, spontaneous viral clearance and response to PEG-INF/RBV therapy, as well as replicating previous findings in a novel population of different ethnic background, an association study was performed analysing *MBL2* polymorphisms in a group of HCV infected patients and healthy controls from North-East of Italy.

Additionally a meta-analysis was performed to unravel the possible role of *MBL2* genetic variants in HCV infection

**Materials and methods**

Study population

Two hundred and three patients were enrolled at the unit "Clinica Medica", Cattinara Hospital and "Italian Liver Foundation" Trieste (Italy): 117 presented with HCV positive chronic hepatitis, 7 with spontaneous HCV clearance (SVC) and 79 patients that cleared the infection after therapy (European-Caucasian, 87 woman and 116 man, mean age=62, standard deviation=14.23, range=26-90).

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Among the HCV chronic patients, the inclusion criteria were: chronic hepatitis with histological diagnosis, HCV-RNA positive with a PCR test, alanine transaminase higher than 1.5 times in the last 6 months, age greater of 18 years; criteria of exclusion from the study were: no hepatic lesions, hepatocellular carcinoma, HIV co-infection, Hepatitis B surface antigen positivity, alcoholic or drug abusers, autoimmunity disorder (serum autoantibody), psychiatric disease with pharmacological treatment, anaemia, alfa1antitrispina deficit, Wilson disease.

All patients have been diagnosed hepatitis C infection through HCV RNA detection (COBAS Ampliprep / COBAS Taqman HCV Test, Roche, Basel, Switzerland) and serological analysis of anti-HCV antibody using enzyme immunoassay and confirmed with recombinant immunoblot assay (Ortho Diagnostic Systems, Raritan, NJ, USA). All patients were seronegative for hepatitis B and HIV serological markers.

HCV RNA genotyping was conducted with the INNO-LiPA HCV Genotyping kit, (Innogenetics)
One-hundred and sixty-tree patients followed the standard therapy that consisted of peghylated Interferon plus ribavirin: patients with HCV genotype type 2 and 3: peghylated interferon 180 μg/kg weekly, ribavirin 800-1200 mg daily, weight dependent, for 24 weeks, patients with genotypes 1 and 4, peghylated interferon 180 μg/kg weekly, ribavirin 800-1200 mg daily, weight dependent for 48 weeks. The patients underwent periodical examinations after 1, 7, 14, 28 day and then every 4 weeks. Treatment response was defined as sustained virological responder (SVR) if patients presented HCV RNA negativity during therapy and maintained this conditions in the follow-up 6 months after the end of treatment; no responder (NR) when the therapy was not effective to eliminate the virus.

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As healthy controls 61 healthy blood donor subjects (European-Caucasian, 32 woman and 29 man, mean age=45, standard deviation=9.88, range=22-67), known to be negative for HCV infection were enrolled at the "Centro Trasfusionale" of "Maggiore Hospital", Trieste (Italy).

A written free and informed consent was obtained from all subjects. All study experiments and procedures have been performed in accordance with the ethical standards of the 1975 Declaration of Helsinki ($6^{th}$ revision, 2008). IRCCS Burlo Garofolo Ethical Committee approved the research project (CIB protocol L.-1055 N. 118/10).

DNA Extraction

Patients' genomic DNA was extracted from whole blood using the DNA extractor NucliSENS® easyMAG® 2.0 (bioMérieux, Marcy l'Etoile, France) instrument according to the manufacturer's protocol.

*MBL2* genotyping

*MBL2* polymorphisms have been genotyped using TaqMan SNPs genotyping assay and TaqMan GTXpress™ Master Mix on ABI7900HT Real Time PCR platform (Applied Biosystems - Life Technologies, Carlsbad, California, U.S.A.) following manufacturer's instructions.

Four *MBL2* SNPs were genotyped using TaqMan pre developed SNPs genotyping assays: C__27858274_10 for promoter XY polymorphism (rs7096206); C___2336610_10 for exon 1 52C>T polymorphism (rs5030737); C___2336609_20 for exon 1 54G>A polymorphism (rs1800450) and C___2336608_20 for exon 1 57G>A polymorphism (rs1800451). For the promoter HL polymorphism (rs11003125), a custom TaqMan SNP genotyping assay was developed. Allelic discrimination was done both manually and automatically with the SDS detection software version 2.1 (Applied Biosystems). The specificity and sensitivity of the Taqman assays were double-checked in blind on 50 DNAs with known *MBL2* genotype: Taqman genotyping results were 100% concordant with the sequenced samples.

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For the analysis, *MBL2* polymorphisms were considered singularly, but also grouping together the three variants on *MBL2* exon 1 as O allele, whereas the wild allele was termed A. The promoter-exon 1 combined genotypes were associated with the protein level and were divided into high producer (HYA/HYA, HYA/LYA, LYA/LYA, HYA/LXA, LYA/LXA, referred as HP), low producer (LXA/LXA, HYA/O, LYA/ O, referred as LP) and deficient producer (LXA/O, O/O, referred as DP) as suggested by Garred et al. [21].

Statistical analysis

Allele, genotype and haplotype frequencies of *MBL2* polymorphisms were calculated by direct counting. The Fisher's exact test for pairwise comparison of allele, genotype and haplotype frequencies (using contingency tables as appropriate) was performed with open-source R version 3.1.3 [22], the adjustment for age and sex was performed with SNPassoc package for R [23], only p-values <0.05 were considered to be significant. *Post-hoc* power calculations were performed with G*Power software version 3.1.9.2 [24].

Meta-analysis

The research of previous published articles was performed within PubMed database and the following key words were used: hepatitis C and mannose binding lectin. Analysis of *MBL2* haplotypes (YA, XA and YO) in HCV infection susceptibility was the inclusion criteria. The analysis was performed with Metafor package [25] for R: strength of association was assessed by the odds ratio (OR) and 95% confidence intervals (CI), the OR was calculated using YA haplotype as references (YA vs. XA and YA vs. YO).

The ORs were analyzed using the random-effects model [26], if the heterogeneity test result was p-value<0.1 or, if the heterogeneity test result was p-value>0.1, the fixed effects model was used [27].

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## Results

Study population

Among the 203 HCV infected individuals enrolled for the study, 141 underwent the PEG-IFN/RBV therapy and 62 did not. Among the treated patients, 79 were SVR and 62 were NR, among the untreated patients 7 spontaneously cleared the virus (SVC), while 55 did not (NSVC).

Patients were then stratified according to their HCV genotype, as infected by genotype 1 (79 among treated individuals; 47 among untreated patients) or infected by genotypes other than 1 (genotypes 2, 3 or 4) (62 among treated subjects; 15 among untreated patients).

All the groups and subgroups were in Hardy-Weinberg equilibrium for *MBL2* polymorphisms analysed.

*MBL2* polymorphisms

The comparison of *MBL2* allelic and genotypic frequencies between the totalities of HCV infected individuals and healthy controls, are reported in table 1: no statistically significant results have been found, even when patients were divided in subjects infected with HCV genotype 1 or not 1 (data not shown).

*MBL2* allelic and genotypic frequencies were then confronted between sustained virus responder (SVR) and non responder (NR) individuals, without finding any statistically significant difference (table 2), also when stratifying for HCV genotype (table 3 and 4) with the exception of O allele and O/O genotype, significantly more frequent in SVR than NR in HCV genotype 1 infected individuals (p=0.01; CI=0.15-0.82; OR=0.35; p=0.03; CI=0.002-1.03; OR=0.10) (table 4) (power analysis=1.00) and after adjustment for age and sex the statistical significance remained (p=0.04, CI=0.01-0.95, OR=0.10) (data not shown).

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No statistically significant associations were observed by comparing SVC and patients with no SVC, even when considering the HCV genotype (data not shown).

Finally, five common *MBL2* haplotypes were found, (HYA, LYA, LXA, HYO, LYO), further arranged in combined genotypes and grouped according to the corresponding MBL production [28]. The HYO haplotype was associated with SVR in treated patients (p=0.01; CI=001-0.65; OR=0.14) (table 2) (power analysis =1.00), also when considering only HCV genotype 1 infected individuals (p=0.02; CI=0.00-0.84; OR=0.10) (table 3) (power analysis >0.99). Similarly, also the deficient producer (DP) combined genotypes were associated with SVR in treated patients (p=0.04; CI=0.09-0.94; OR=0.32) (table 2) (power analysis >0.99) and after adjustment for age and sex the statistical significance became stronger (p=0.01, CI=0.06-0.60, OR=0.19) (data no shown).

Meta-analysis

Only three studies [17, 19, 29] agreed with the inclusion criteria and showed usable data: they inquired the association between HCV infection susceptibility and *MBL2* functional haplotypes. The table 5 reports the basic characteristic of the studies. For the comparison between YA and XA haplotypes, the test for heterogeneity presented a p-value>0.05, therefore the Fixed Effects Model was chosen and it showed an association between the subjects carrying YA haplotype and protection against HCV infection (OR=0.75, CI=0.59-0.96) (figure 1), instead considering the YA and YO haplotype the test of heterogeneity had a p-value=0.03, so the Random Effect Model was used but no statistically significant results were observed (OR=0.84, CI=0.55-1.27) (figure 2).

Although in the present study the haplotype power analysis was low (0.33) in the comparison between healthy controls and HCV patients, data from the current work were also included in the meta-analysis, but the statistical significance was lost (YA vs XA: test for heterogeneity p-value>0.05, Fixed Effects Model, OR=0.83, CI=0.59-1.16; YA vs YO: test for heterogeneity p-value=0.04, Random Effect Model, OR=0.88, CI=0.63-1.24).

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**Discussion**

Although MBL innate immunity molecule has been reported as possessing anti-viral activity against HCV in our study we did not find ~~an~~ association between functional *MBL2* polymorphisms and susceptibility to be infected by HCV, in agreement with previous genetic studies [29-32], performed on ethnic groups different from the Italian one here analysed; moreover Killpatrick et al. described a lack of association between circulating serum levels of MBL and susceptibility towards hepatitis C infection [33]. On the other hand, three other studies have suggested an association of *MBL2* genetic variants with HCV infection susceptibility [17-19]. The differences encountered among the works mentioned above could be explained on the basis of different ethnic origin of the individuals analysed, being *MBL2* polymorphisms frequencies different among populations [34], as well as considering the low number of patients and controls enrolled in all studies, this work included, finally taking into account the lack of an appropriate control group (with the same risk exposure of the infected patients).

A meta-analysis was performed with data concerning three different populations (Euro-Brazilian, Brazilian and Japanese) from three studies [17-19, 29], the *MBL2* YA haplotype was associated with HCV infection susceptibility compared to XA but not to YO haplotype; these results counteracted the negative findings of the current study (data not shown), however, when the results of the present study on Caucasian subjects were included in the meta-analysis the statistical significance was lost.

Consequently it is possible to speculate that *MBL2* polymorphisms might be not involved in HCV infection susceptibility, or if the association was present, was weak.

In this study *MBL2* polymorphisms were not associated with spontaneous viral clearance but besides no previous study investigated this issue, however only seven subjects eliminated naturally virus and this number is too low to provide enough statistical power supporting the role of *MBL2* genetic variants in the context of virus clearance.

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Then considering the response to interferon treatment, an association was found between *MBL2* polymorphisms and SVR: the HYO haplotype and DP combined genotype were associated to SVR in treated individuals; in addition to HYO haplotypes, also *MBL2* O/O genotype and O allele (correlated with low MBL serum levels [28]) were associated with SVR in individuals infected with genotype 1 HCV; the statistical power of these significant findings was high and corroborated the results.

MBL has been previously reported as a double-edged sword in the context of HIV viral infection: high level of circulating proteins confer protection against virus infection, but once the virus infect the cell high MBL level are detrimental since enhancing the production of pro-inflammatory cytokines and consequently favouring viral replication [35]. So we can hypothesize a similar scenario for HCV where individuals with low MBL levels, should have a better control of HCV replication, consequently improving their response to the treatment. ~~Similarly to~~ In contradiction with these findings, Matsushita et al. [29] reported in a Japanese population that low producer alleles and haplotypes might be predictors for bad outcomes of interferon therapy, however the different ethnicity and the use of only IFN without RBV on Japanese HCV patients could explain the differences encountered.

Conversely, Killpatrick et al. [33] and Dumestre-Perard et al. [36] didn't show any influence of MBL serum expression on interferon therapy response. Furthermore no association was found between *MBL2* polymorphisms and hepatitis outcome in treated Turkish [30] and Greek [37] HCV patient.

MBL is an acute-phase reactant protein and its serum level would be increased after an acute infection [38], or regulated by interleukins, heat shock, and other biological triggers, some of which are interferon-inducible [39]. Additionally it has been speculated that complement mediated enhanced phagocytosis, as a result of opsonization, could facilitate intracellular infections [40] so in

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some cases, high level of MBL protein could be disadvantageous for infected subjects and supported the pathogen spreading. Finally low level of protein production might moderate a strong complement-mediated activation that could result in tissue damage as observed in inflammatory situation [41]. However, as for susceptibility to HCV, *MBL2* polymorphisms showed controversial results, possibly due to the limited number of samples analyzed and to ethnic differences; we have also to consider that susceptibility to HCV infection as well as the treatment response are multifactorial complex traits with many genes involved, so *MBL2* being just a piece of the puzzle could have a limited influence on the phenotypes.

The genetic factors that influence the natural course of HCV infection and the response to treatment are not yet fully understood [42, 43]. In this work *MBL2* gene polymorphisms were analysed as potential factor involved in HCV infection susceptibility and therapy outcome. *MBL2* polymorphisms seemed to interfere marginally with interferon therapy response, and the literature showed concordant and discordant results with the present study. So, considering the main limitation of association analyses comparing the distribution of *MBL2* polymorphisms in HCV infected individuals and healthy controls or between subjects with different response to interferon therapy, related to the low number of subjects enrolled and different ethnic background, only a collaborative study enrolling greater number of patients and control, such as those of GWAS, could disclose the true role of *MBL2* in the modulation of these complex traits. If the association between *MBL2* functional genetic variants and SVR will be confirmed, we can figure out the introduction of *MBL2* genotyping, at present fully optimized and feasible by laboratory technicians in mass scale, in the clinical routine follow-up of HCV infected patients.

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**Author's contributions**

All authors contributed equally to the work: LZ performed *MBL2* genotyping, conducted the statistical analyses and drafted the manuscript; VP participated in writing the manuscript; GA extracted the DNA samples; GM collected the samples and participated in patients follow-up; GP, LSC and FM were responsible for the setting of the clinical protocol and management of patients; SC critically revised the manuscript; LS conceived the study and revised the manuscript.

**Conflict of interest**

The authors declared no conflict of interest

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Table 1: *MBL2* polymorphism allele, genotype and haplotype counts (and frequencies) in HCV infected patients (HCV) and healthy controls (HC)

| HCV infection | HCV<br>n=203 | HC<br>n=61 | HCV vs. HC |
|---|---|---|---|
| *MBL2* H/L | | | |
| L | 272 (0.67) | 78 (0.64) | ref |
| H | 134 (0.33) | 44 (0.36) | p=0.58; CI=0.56-1.37; OR=0.87 |
| L/L | 87 (0.43) | 23 (0.38) | ref |
| H/L | 98 (0.48) | 32 (0.52) | p=0.54; CI=0.42-1.55; OR=0.81 |
| H/H | 18 (0.09) | 6 (0.10) | P=0.78; CI=0.26-2.74; OR=0.79 |
| HWE | $\chi^2$=1.70; p=0.19 | $\chi^2$=1.15; p=0.28 | |
| *MBL2* X/Y | | | |
| Y | 302 (0.74) | 92 (0.75) | ref |
| X | 104 (0.26) | 30 (0.25) | p=0.91; CI=0.65-1.75; OR=1.06 |
| Y/Y | 110 (0.54) | 34 (0.56) | ref |
| X/Y | 82 (0.40) | 24 (0.39) | p=0.88; CI=0.56-2.01; OR=1.06 |
| X/X | 11 (0.05) | 3 (0.05) | p=1.00; CI=0.28-6.68; OR=1.13 |
| HWE | $\chi^2$=0.73; p=0.39 | $\chi^2$=0.23; p=0.63 | |
| *MBL2* AO | | | |

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| | | | |
|---|---|---|---|
| A | 320 (0.79) | 95 (0.78) | ref |
| O | 86 (0.21) | 27 (0.22) | p=0.80; CI=0.57-1.61; OR=0.95 |
| A/A | 128 (0.63) | 39 (0.64) | ref |
| A/O | 64 (0.31) | 17 (0.28) | p=0.75; CI=0.58-2.34; OR=1.15 |
| O/O | 11 (0.05) | 5 (0.08) | p=0.54; CI=0.1920-2.62; OR=0.67 |
| HWE | $\chi^2$=0.63; p=0.43 | $\chi^2$=2.23; p=0.13 | |
| MBL production | | | |
| HP | 117 (0.58) | 36 (0.59) | ref |
| LP | 50 (0.25) | 16 (0.26) | p=1.00; CI=0.50-2.03; OR=0.96 |
| DP | 36 (0.18) | 9 (0.15) | p=0.69; CI=0.52-3.18; OR=1.23 |
| Haplotypes | | | |
| HYA | 113 (0.28) | 37 (0.30) | ref |
| LXA | 104 (0.26) | 30 (0.25) | p=0.68; CI=0.63-2.05; OR=1.13 |
| LYA | 106 (0.26) | 28 (0.23) | p=0.48; CI=0.68-2.26; OR=1.24 |
| LYO | 62 (0.15) | 20 (0.16) | p=1.00; CI=0.52-2.01; OR=1.01 |
| HYO | 21 (0.05) | 7 (0.06) | p=1.00; CI=0.36-2.96; OR=0.98 |

Abbreviations: HCV = Hepatitis C virus; HC = healthy controls; MBL = mannose binding protein C; HWE = Hardy Weinberg equilibrium; HP = high producer (HYA/HYA, HYA/LYA, LYA/LYA, HYA/LXA, LYA/LXA); LP = low producer (LXA/LXA, HYA/O, LYA/ O); DP = deficient producer (LXA/O, O/O)

Table 2: *MBL2* polymorphism allele, genotype and haplotype counts (and frequencies) in HCV infected patients treated with IFN therapy classified as sustained virological responder (SVR) and non responder (NR).

| HCV infection | NR | SVR | NR vs. SVR |
|---|---|---|---|
| | n=62 | n=79 | |
| *MBL2* H/L | | | |
| L | 81 (0.65) | 101 (0.64) | ref |
| H | 43 (0.34) | 57 (0.36) | p=0.90; CI=0.56-1.58; OR=0.94 |
| L/L | 25 (0.40) | 31 (0.39) | ref |
| H/L | 31 (0.50) | 39 (0.50) | p=1.00; CI=0.47-2.18; OR=1.01 |
| H/H | 6 (0.10) | 9 (0.11) | p=1.00; CI=0.22-2.98; OR=0.84 |
| HWE | $\chi^2$=0.67;p=0.41 | $\chi^2$=0.39; p=0.53 | |
| *MBL2* X/Y | | | |
| Y | 94 (0.76) | 115 (0.73) | ref |
| X | 30 (0.24) | 43 (0.27) | p=0.59; CI=0.48-1.51; OR=0.85 |
| Y/Y | 36 (0.58) | 41 (0.52) | ref |
| X/Y | 22 (0.35) | 33 (0.42) | p=0.48; CI=0.35-1.62; OR=0.76 |
| X/X | 4 (0.06) | 5 (0.06) | p=1.00; CI=0.17-4.60; OR=0.91 |
| HWE | $\chi^2$=0.06; p=0.80 | $\chi^2$=0.23; p=0.63 | |
| *MBL2* AO | | | |

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| | | | |
|---|---|---|---|
| A | 103 (0.83) | 116 (0.73) | ref |
| O | 21 (0.17) | 42 (0.27) | p=0.06; CI=0.30-1.05; OR=0.56 |
| A/A | 43 (0.69) | 44 (0.56) | ref |
| A/O | 17 (0.27) | 28 (0.35) | p=0.27; CI=0.28-1.37; OR=0.62 |
| O/O | 2 (0.03) | 7 (0.09) | p=0.17; CI=0.03-1.67; OR=0.29 |
| HWE | $\chi^2$=0.04; p=0.84 | $\chi^2$=0.67; p=0.41 | |
| MBP production | | | |
| HP | 39 (0.63) | 39 (0.49) | ref |
| LP | 17 (0.27) | 21 (0.27) | p=0.69; CI=0.34-1.89; OR=0.81 |
| DP | 6 (0.10) | 19 (0.24) | p=0.04; CI=0.09-0.94; OR=0.32 |
| Haplotypes | | | |
| HYA | 41 (0.33) | 42 (0.27) | ref |
| LXA | 32 (0.26) | 43 (0.27) | p=0.43; CI=0.39-1.50; OR=0.76 |
| LYA | 33 (0.27) | 33 (0.21) | p=1.00; CI=0.51-2.06; OR=1.02 |
| LYO | 16 (0.13) | 25 (0.16) | p=0.34; CI=0.28-1.49; OR=0.66 |
| HYO | 2 (0.02) | 15 (0.09) | p=0.01; CI=0.01-0.65; OR=0.14 |

Abbreviations: HCV = Hepatitis C virus; NR = non responder; SVR= sustained virological responder; MBL = mannose binding protein C; HWE = Hardy Weinberg equilibrium; HP = high producer (HYA/HYA, HYA/LYA, LYA/LYA, HYA/LXA, LYA/LXA); LP = low producer (LXA/LXA, HYA/O, LYA/ O); DP = deficient producer (LXA/O, O/O)

Table 3: *MBL2* polymorphism allele, genotype and haplotype counts (and frequencies) in genotype 1 HCV infected patients treated with IFN therapy classified as sustained virological responder (SVR) and non responder. (NR).

| HCV genotype 1 infection | NR | SVR | NR vs. SVR |
|---|---|---|---|
| | n=46 | n=33 | |
| *MBL2* H/L | | | |
| L | 59 (0.64) | 38 (0.58) | ref |
| H | 33 (0.36) | 28 (0.42) | p=0.41; CI=0.38-1.53; OR=0.76 |
| L/L | 18 (0.39) | 11 (0.33) | ref |
| H/L | 23 (0.50) | 16 (0.48) | p=1.00; CI=0.29-2.62; OR=0.88 |
| H/H | 5 (0.11) | 6 (0.18) | p=0.48; CI=0.10-2.59; OR=0.52 |
| HWE | $\chi^2$=0.35; p=0.57 | $\chi^2$=0.002; p=0.96 | |
| *MBL2* X/Y | | | |
| Y | 69 (0.75) | 52 (0.79) | ref |
| X | 23 (0.25) | 14 (0.21) | p=0.70; CI=0.55-2.87; OR=1.24 |
| Y/Y | 26 (0.56) | 21 (0.64) | ref |
| X/Y | 17 (0.36) | 10 (0.30) | p=0.63; CI=0.47-4.10; OR=1.37 |
| X/X | 3 (0.07) | 2 (0.06) | p=1.00; CI=0.12-15.70; OR=1.21 |
| HWE | $\chi^2$=0.01; p=0.92 | $\chi^2$=0.29; p=0.59 | |
| *MBL2* AO | | | |

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| | | | |
|---|---|---|---|
| A | 79 (0.86) | 45 (0.68) | ref |
| O | 13 (0.14) | 21 (0.32) | p=0.01; CI=0.15-0.82; OR=0.35 |
| A/A | 34 (0.74) | 17 (0.51) | ref |
| A/O | 11 (0.24) | 11 (0.33) | p=0.20; CI=0.16-1.57; OR=0.50 |
| O/O | 1 (0.02) | 5 (0.15) | p=0.03; CI=0.002-1.03; OR=0.10 |
| HWE | $\chi^2$=0.01; p=0.92 | $\chi^2$=1.77; p=0.18 | |
| MBP production | | | |
| HP | 31 (0.67) | 15 (0.45) | ref |
| LP | 12 (0.26) | 12 (0.36) | p=0.20; CI=0.16-1.50; OR=0.49 |
| DP | 3 (0.07) | 6 (0.18) | p=0.07; CI=0.03-1.35; OR=0.25 |
| Haplotypes | | | |
| HYA | 32 (0.35) | 21 (0.32) | ref |
| LXA | 23 (0.25) | 14 (0.21) | p=1.00; CI=0.42-2.81; OR=1.08 |
| LYA | 25 (0.26) | 12 (0.18) | p=0.51; CI=0.52-3.66; OR=1.36 |
| LYO | 11 (0.12) | 12 (0.18) | p=0.33; CI=0.20-1.81; OR=0.61 |
| HYO | 1 (0.01) | 7 (0.10) | p=0.02; CI=0.00-0.84; OR=0.10 |

Abbreviations: HCV = Hepatitis C virus; NR = non responder; SVR= sustained virological responder; MBL = mannose binding protein C; HWE = Hardy Weinberg equilibrium; HP = high producer (HYA/HYA, HYA/LYA, LYA/LYA, HYA/LXA, LYA/LXA); LP = low producer (LXA/LXA, HYA/O, LYA/ O); DP = deficient producer (LXA/O, O/O)

Table 4: *MBL2* polymorphism allele, genotype and haplotype counts (and frequencies) in genotypes other than 1 HCV infected patients treated with IFN therapy classified as sustained virological responder (SVR) and non responder (NR).

| HCV genotype other than 1 infection | NR | SVR | NR vs. SVR |
|---|---|---|---|
| | n=16 | n=46 | |
| *MBL2* H/L | | | |
| L | 22 (0.69) | 63 (0.68) | ref |
| H | 10 (0.31) | 29 (0.32) | p=1.00; CI=0.37-2.52; OR=0.99 |
| L/L | 7 (0.44) | 20 (0.43) | ref |
| H/L | 8 (0.50) | 23 (0.50) | p=1.00; CI=0.26-3.86; OR=0.99 |
| H/H | 1 (0.06) | 3 (0.07) | p=1.00; CI=0.01-14.38; OR=0.95 |
| HWE | $\chi^2$=0.43; p=0.51 | $\chi^2$=1.15; p=0.28 | |
| *MBL2* X/Y | | | |
| Y | 23 (0.72) | 63 (0.68) | ref |
| X | 9 (0.28) | 29 (0.32) | p=0.82; CI=0.31-2.21; OR=0.85 |
| Y/Y | 8 (0.50) | 20 (0.43) | Ref |
| X/Y | 7 (0.44) | 23 (0.50) | p=0.77; CI=0.20-2.90; OR=0.76 |
| X/X | 1 (0.06) | 3 (0.07) | p=1.00; CI=0.01-12.39; OR=0.84 |
| HWE | $\chi^2$=0.11; p=0.74 | $\chi^2$=1.15; p=0.28 | |

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| | | | |
|---|---|---|---|
| *MBL2* AO | | | |
| A | 26 (0.81) | 71 (0.77) | ref |
| O | 6 (0.19) | 21 (0.23) | p=0.80; CI=0.23-2.30; OR=0.78 |
| A/A | 11 (0.69) | 27 (0.59) | ref |
| A/O | 4 (0.25) | 17 (0.37) | p=0.54; CI=0.12-2.39; OR=0.58 |
| O/O | 1 (0.06) | 2 (0.04) | p=1.00; CI=0.02-25.79; OR=1.22 |
| HWE | $\chi^2$=0.51; p=0.47 | $\chi^2$=0.11; p=0.74 | |
| MBP production | | | |
| HP | 10 (0.63) | 24 (0.52) | ref |
| LP | 4 (0.25) | 9 (0.20) | p=1.00; CI=0.19-5.05; OR=1.06 |
| DP | 2 (0.12) | 13 (0.28) | p=0.30; CI=0.03-2.18; OR=0.38 |
| Haplotypes | | | |
| HYA | 9 (0.28) | 21 (0.23) | ref |
| LXA | 9 (0.28) | 29 (0.31) | p=0.59; CI=0.21-2.47; OR=0.73 |
| LYA | 8 (0.25) | 21 (0.23) | p=1.00; CI=0.24-3.18; OR=0.89 |
| LYO | 5 (0.16) | 13 (0.13) | p=1.00; CI=0.19-3.83; OR=0.90 |
| HYO | 1 (0.03) | 8 (0.09) | p=0.40; CI=0.01-2.85; OR=0.30 |

Abbreviations: HCV = Hepatitis C virus; NR = non responder; SVR= sustained virological responder; MBL = mannose binding protein C; HWE = Hardy Weinberg equilibrium; HP = high producer (HYA/HYA, HYA/LYA, LYA/LYA, HYA/LXA, LYA/LXA); LP = low producer (LXA/LXA, HYA/O, LYA/ O); DP = deficient producer (LXA/O, O/O)

Table 5: characteristics of the studies included in the meta-analysis

| **Study** | **Ethnicity** | **Patients man/woman** | **age** | **n. controls** | **n. patients** |
|---|---|---|---|---|---|
| Halla et al. | Brazilian | 97/89 | 53 | 232 | 186 |
| Pedroso et al. | Euro-Brazilian | 65/37 | 51 | 102 | 102 |
| Matsushita et al. | Japanese | NA | NA | 218 | 159 |

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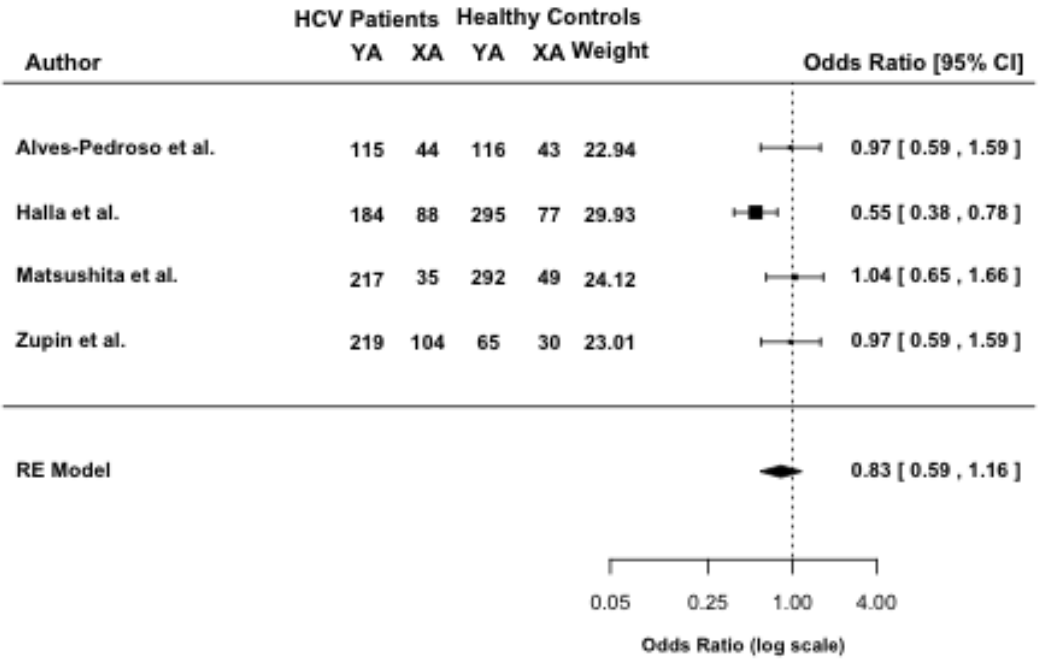


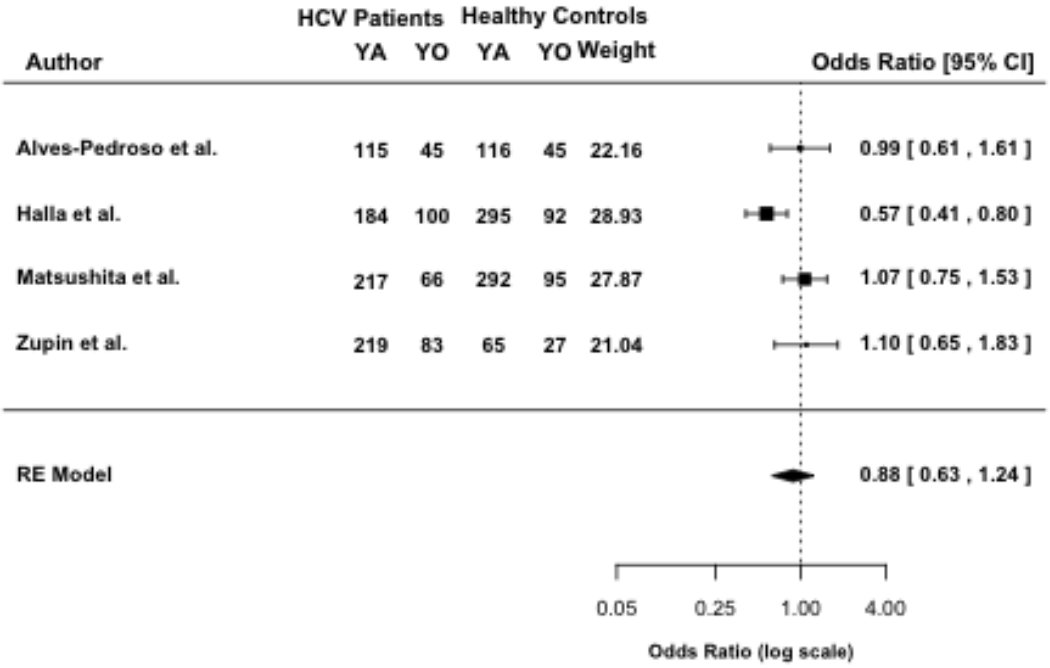


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