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Brief communication

MASP2 gene polymorphism is associated with susceptibility to hepatitis C virus infection

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ABSTRACT

Hepatitis C virus (HCV) has become a major public health issue and is prevalent in most countries. We examined several *MASP2* functional polymorphisms in 104 Brazilian patients with moderate and severe chronic hepatitis C using the primers set to amplify the region encoding the first domain (CUB1), a critical region for the formation of functional mannan-binding lectin (MBL)/MBL-associated serine proteases (MASP)–2 complexes, and the fifth domain (CCP2), which is essential for C4 cleavage of the MASP2 gene. We identified five single nucleotide polymorphisms in patients and controls: p. R99Q, p. D120G, p.P126L, p.D371Y, and p.V377A. Our results show that the p.D371Y variant (c.1111 G > T) is associated with susceptibility to HCV infection ($p = 0.003$, odds ratio = 6.33, 95% confidence interval = 1.85–21.70). Considered as a dominant function for the T allele, this variant is associated with high plasma levels of the MASP-2 in hepatitis C patients ($p < 0.001$). However, further functional investigations are necessary to understand the degree of involvement between *MASP2* and the HCV susceptibility.

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1. Introduction

The hepatitis C virus (HCV) infects approximately 170 million persons worldwide and despite the reduction over the last two decades, has become a major public health issue [1,2]. Liver injury induced by the virus is thought to be mainly immune mediated rather than a result of direct cytopathic effects of the infection [3]. It is estimated that approximately 15% of HCV-infected individuals eliminate the virus spontaneously, whereas 25% develop a mild form of the disease and 60% progress to the chronic form. Fibrosis is the principal complication of chronic hepatitis C, which increases both morbidity and mortality because of complications such as cirrhosis or hepatocarcinoma.

The innate response to HCV slows down viral replication and activates cytokines that trigger the synthesis of antiviral proteins, whereas the adaptive immune system neutralizes virus particles and destroys infected cells [4]. Complement proteins are involved in the early innate responses against pathogens and play a role in clearing circulating viral antigens from the blood of infected hosts.

Mannan-binding lectin (MBL) is a collectin, encoded by the *MBL2* gene, that can act directly as opsonin or activate MBL-associated serine proteases (MASPs) initiating an antibody-independent pathway of complement activation [5]. Three MASPs have been described: MASP-1, -2, and -3. MASP-2 plays the predominant functional role in the activation of the lectin pathway [6], whereas MASP-2 forms complexes with the pattern recognition molecules MBL and ficolins (H-ficolin, L-ficolin, or M-ficolin) and is activated when one of these proteins recognizes micro-organisms leading to the subsequent cleavage of C4 and C2, thus initiating complement cascade activation [7,8]. HCV encodes two highly glycosylated envelope proteins, E1 and E2, which are potential targets for interaction with MBL and ficolins [9].

MASP-2 is encoded by the *MASP2* gene in the chromosome 1p36.23–31. The mRNA is encoded by 11 exons, of which a single exon encodes the linker region and the serine protease domain [10]. The MASP-2 protein is composed of six modules and a linker region: an N-terminal CUB domain (CUB1) followed by epidermal growth factor (EGF)–like domain of Ca^{2+} binding type, a second CUB domain (CUB-2), 2 contiguous complement control protein modules (CCP1 and CCP2), a short linker, and finally, a chymotrypsin-like serine protease (SP) domain. The interaction with MBL and L-ficolin is determined by N-terminal CUB1-EGF moiety but is strengthened by the presence of the CUB2 module [11]. Two complement control protein (CCP) molecules and the SP domain perform the catalytic activity of the entire molecule and CCP2 retains the essential role in C4 cleavage [12].

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MBL deficiency, occurring at a frequency of about 2–10%, is the most common congenital immunodeficiency, and is associated with susceptibility to infection and autoimmune disorders [13]. Inherited MASP-2 deficiency has been described as the result of a mutation causing the exchange of aspartic acid with glycine at the 105th position in the first domain CUB1, involved in calcium binding. This mutation prohibits binding to MBL and ficolins, and deprives MASP-2 of functional activity. The frequency of this gene mutation among Caucasian individuals is 3.6% [11].

Several naturally occurring polymorphisms of the *MASP2* gene have been described in different populations. Nine single nucleotide polymorphisms (SNPs) were observed: p. R99Q, p. R118C, p.D120G, p.P126L, p.H155R, p.156_159dupCHNH, p.D371Y, p.V377A, and p.R439H [7]. Thiel et al. (2007) [7] reported a reduction in MASP-2 levels in individuals with a p.V377A mutation. More recently, the same group observed that when both p.D120G and p.156_159CHNHdup were present, the protein was misfolded and therefore unable to associate with MBL [8].

Mutant *MBL2* haplotypes have been linked to disease progression and response to therapy in HCV infection [9,14]; however, the impact of *MASP2* polymorphisms on the susceptibility to HCV infection has not yet been addressed.

Although the mechanisms responsible for development of severe HCV disease in some patients are not understood, it is likely that viral as well as host immune factors play an important role in the process. In the present study, we examined several *MASP2* functional polymorphisms in Brazilian patients with moderate and severe chronic hepatitis C, defined by low and high fibrosis stage scores, using the primers set to amplify the region encoding the first domain (CUB1) and fifth domain of the *MASP2* gene.

2. Subjects and methods

2.1. Patients

A total of 104 patients (72 men and 32 women) were studied, with a mean (standard deviation) age of 49 ±10 years, from the city of Curitiba in southern Brazil (Supplementary Table S1) informed consent was obtained from all participants, and the study was approved by the institutional review boards of participating centers. All patients had positive anti-HCV atibodies by enzyme-linked immunosorbent assay (ELISA) and positive viral RNA using reverse transcription–polymerase chain reaction (Amplicor HCV, version 2.0; Roche, Branchburg, NJ). Only patients more than 18 years of age and with stage F2 or higher liver fibrosis according to the Metavir classification were included [15]. The exclusion criteria were as follows: alcohol use (>20 g or 40 g daily for women and men, respectively), co-infection with hepatitis B virus or human immunodeficiency virus (HIV), and presence of any other liver or systemic disease. Most patients were treated with conventional interferon-α (IFN-α) or with pegylated_IFN-α (pgIFN) associated with ribavirin. *MBL2* genotyping was determined previously using a real-time polymerase chain reaction with fluorescent hybridization probes [14]. A gender-, ethnicity, and age-matched control group was composed of 104 healthy individuals (Supplementary Table S2).

2.2. MASP-2 concentration

The concentration of MASP-2 in the patients was estimated by a time-resolved immunofluorometric assay (TRIFMA). The TRIFMA is a sandwich-type method using a combination of two monoclonal anti-MASP-2 antibodies for quantifying MASP-2 in serum. The serum dilution applied (1/50) allows detection of between 10 ng and 10 μg MASP-2/ml serum.

2.3. Polymerase chain reaction–single strand conformation polymorphism

The exons of the gene encoding MAP 19 and MASP-2 were analyzed using polymerase chain reaction (PCR), the primer sets 5′GCGAGTACGACTTCGTCAAGG3′ and 5′CTCGGCTGCATAGAAGGCCTC3′ to amplify a parts of the region encoding the first domain (CUB1) and 5′CCAGACCTTTGGAAAGTTAGC3′ and 5′GGCTCAAGTTCCAAGTATTGC3′ to amplify part of the region encoding the fifth domain (the complement control protein domain 2 of MASP-2-CCP2; exons 3 and 9, respectively). The CUB domains are critical for the formation of functional MBL/MASP-2 complexes. The CCP2 domain is essential for C4 cleavage.

The cycle conditions were as follows: denaturation of the template DNA for one cycle of 95°C for 120 seconds; and amplification of the target DNA for 40 cycles of 94°C for 15 seconds, 54°C for 15 seconds, and 72°C for 30 seconds. PCR was followed by mutation screening via single strand conformation polymorphism (SSCP) as previously described [16]. When abnormal band patterns were detected under SSCP analysis, direct DNA sequencing was carried out to confirm the alleles. Genotype and allele frequencies were obtained by direct counting.

2.4. Statistical analysis

The allele frequency distributions of the patient and control populations were compared by Fisher's exact test. Possible associations between *MASP2* genotypes or alleles and susceptibility to severe disease were analyzed with χ^2 test and Fisher's exact test (Statistica v8.0). The parametric Student's *t* test was used for quantitative data. MASP-2 levels and genotypes were compared between the groups using ANOVA and the least significant difference test. The level of significance was set at $p < 0.05$.

2.5. Linkage disequilibrium

Linkage disequilibrium was measured by r^2 values using Haploview 4.0. software. The range of r^2 was from 0 to 1, and linkage disequilibrium was considered for marker pairs with $r^2 > 0.8$.

With regard to *in silico* analyses, the MASP2 aminoacid sequence isoform 1 (O00187-1) used in this work was obtained from UniProtKB/Swiss-Prot version 133. The in silico functional analysis of each SNP was performed through the program "PolyPhen: prediction of functional effect of human nsSNPs" (http://genetics.bwh.harvard.edu/pph/). The main databases used by the program include all human SNPs from dbSNP build 126; and Protein Quaternary Structure (PQS), Protein Data Bank (PDB), and Dictionary of Secondary Structure in Proteins (DSSP) as structural database [17,18].

3. Results

We identified five SNPs in both patient and control groups: p.R99Q (c.296 G > A), p.D120G (c.359 A > G), p.P126L (c.377 C > T), p.D371Y (c.1111 G > T), and p.V377A (c.1130 T > C) (Table 1). All allele and genotype frequencies were consistent with those expected according to the Hardy–Weinberg equilibrium, and linkage disequilibrium was not detected among these five *MASP2* polymorphisms ($r^2 < 0.8$). Gene frequencies of *MASP2* variants occurred according to previous reports on Caucasian populations [7].

Additional *in silico* analyses of these SNPs indicated that only p.D120G (c.359A > G) was predicted to cause damage to the MASP-2 protein. This variant showed a profile score for the amino acid substitution (PSIC) of 2.817, and the evolutionary preservation of residue D can be seen in the corresponding position of different species (Table 2). An evolutionary preservation was also observed in the p.D371Y (c.1111G > T) variation, for which conservation was seen in three of four groups of species analyzed. However, the

Table 1
Distribution of frequency of SNPs in *MASP-2* genotyping among HCV patients

Allele	SNP ID	mRNA	Genotype	HCV patients (%)	Controls (%)	*p* Value[a]
p. R99Q	rs61735600	c.296 G > A	GG	103 (99.04)	104 (100.00)	1.0
			GA	1 (0.96)	0 (0.00)	
			AA	0 (0.00)	0 (0.00)	
			AA + GA	1 (0.96)	0 (0.00)	
p.D120G	rs72550870	c.359 A > G	GG	0 (0.00)	0 (0.00)	1.0
			GA	2 (1.92)	2 (1.92)	
			AA	102 (98.08)	102 (98.08)	
			GG + AG	2 (1.92)	2 (1.92)	
p. P126L	rs56392418	c.377 C > T	CC	103 (99.04)	101 (97.12)	0.621
			CT	1 (0.96)	3 (2.88)	
			TT	0 (0.00)	0 (0.00)	
			TT + CT	1 (0.96)	3 (2.88)	
p.D371Y	rs12711521	c.1111 G > T	GG	16 (15.53)	4 (3.92)	0.008
			GT	35 (33.98)	47 (46.08)	
			TT	52 (50.49)	51 (50.00)	
			TT + GT	87 (84.47)	98 (96.08)	
p. V377A	rs2273346	c.1130 T > C	TT	97 (94.17)	93 (91.18)	0.436
			TC	6 (5.83)	8 (7.84)	
			CC	0 (0.00)	1 (0.98)	
			CC + TC	6 (5.83)	9 (8.82)	

[a]For *p* values, the results of the better genetic models are reported when the codominant and dominant models were tested by means of univariated analysis using the Fisher's exact test and χ^2 test ($p < 0.05$).

profile score for this variation did not represent a harmful effect (Table 2).

Upon comparison of genotype frequency between patients and controls, only the p.D371Y variant (c.1111 G > T) reached statistical significance with genotype *GG* significantly increasing the susceptibility to HCV infection ($p = 0.003$, odds ratio [OR] = 6.33; 95% confidence interval [CI] = 1.85–21.70).

Because of the low frequency of the other polymorphisms, MASP-2 levels were compared only with c.1111G > T and c.1130T > C. A significant association of MASP-2 concentration was observed in the presence of the allele c.1111G > T (range, 24.0 – 886.0 ng/ml). When the dominant model to G at c.1111G > T was tested, MASP-2 levels were significantly higher in *GG* and *GT* genotypes in HCV patients ($p < 0.001$; Fig. 1).

Analysis of the response to IFN with ribavirin treatment at positions c.1111G > T and c.1130T > C, showed that eight (33.3%) of the 15 patients at position c.1111G > T and 9 (60.0%) at c.1130 T > C were nonresponders. No statistical significance was observed between the two groups ($p = 0.565$ and $p = 0.489$, respectively).

No association was found between genotype variants c.1111G > T or c.1130T > C, and degree of liver fibrosis ($p = 0.087$ and $p = 0.070$, respectively).

4. Discussion

In this study, we investigated *MASP2* functional polymorphisms in Euro-Brazilian patients with moderate and severe chronic hepatitis C. MASP-2 is an important molecule in the activation of the complement system via MBL and ficolins [11]. We demonstrated that the p.D120G variant (c.359A > G) was not associated with HCV infection. Recent studies reported that this *MASP-2* mutation was associated with low MASP-2 levels and functional deficiency in Caucasian individuals, and only those individuals who are homozygous for the p.D120G mutation have no ability to activate the MBL pathway [19].

Thiel et al. (2009) [8] previously described that the presence of the *MASP2* p.D120G variant resulted in a misfolded protein that was unable to associate with MBL and subsequently activate C4.

By contrast, our results show that the p.D371Y variant (c.1111 G > T) is associated with the susceptibility to HCV infection. Considering a dominant function for the T allele, this variant is associated with high plasma levels of the MASP-2 in hepatitis C patients. Although the complement cascade activation in the presence of the p.D371Y variant (c.1111 G > T) was not analyzed in the present study, we speculate that in individuals homozygous for this polymorphism, MASP-2 may be less functional. It is possible that the amino acid change in this position causes modification in the catalytic region of the enzyme, altering the substrate specificity. Although the conversion of aspartic acid to tyrosine in p.D371Y (c.1111 G > T) can be accommodated in the molecule without disturbing its overall structure, it is possible that this variation has a functional effect in response to HCV infection. Thiel et al. (2007) [7] reported that the p.V377A allele (c.1130T > C) influenced MASP-2 levels and that the difference among homozygous individuals was significant. Boldt et al. (2011) [20] reported that the p.D371Y and p.V377A variants were associated with varying MASP-2 plasma levels in chronic Chagas disease.

Nevertheless, no significant association between alleles or genotypes of *MASP2* p.V377A and p.D371Y or susceptibility to severe

Table 2
In silico modeling of the effect of *MASP-2* missense substitution on the protein function

#	DNA change	Protein change	SNP ID	Domains	*In silico* modeling		Interspecies alignment				
					Prediction	Score[a]	*Homo Sapiens*	*Rattus novergicus*	*Galus Galus*	*Xenopus tropicalis*	*Cyprinus Carpio*
1	c.296 G > A	p. R99Q	Rs61735600	CUBI	Likely benign	0.306	R	R	E	K	D
2	c.359 A > G	p.D120G	rs72550870	CUBI	Probably damaging	2.871	D	D	D	D	D
3	c.377 C > T	p.P126L	rs56392418	CUBI	Likely benign	1.188	P	P	P	E	R
4	c.1111 G > T	p.D371Y	rs12711521	CCP2	Likely benign	1.374	D	D	D	D	E
5	c.1130 T > C	p.V377A	rs2273346	CCP2	Likely benign	1.305	V	V	I	Y	M

[a]Greater scores indicate higher probability to impair the protein function; factors taken into account for the calculation of the score are as follows: difference in thermo-physical properties of the wt and mutant protein; and evolutionary preservation of the residue in the corresponding position.

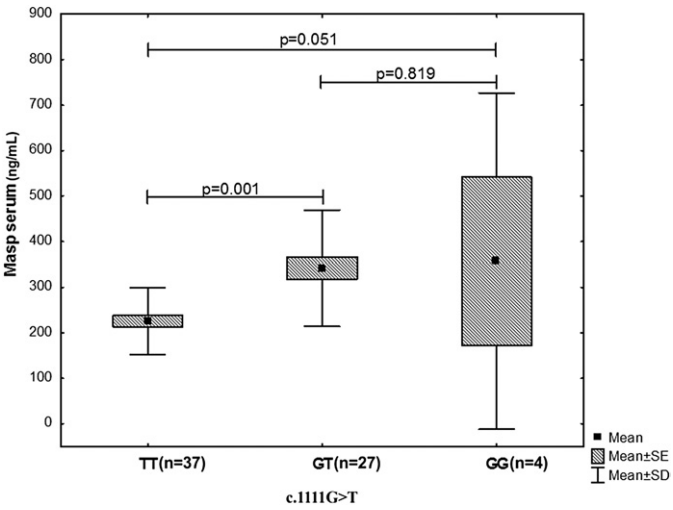

Fig. 1. MASP-2 serum level observed in patients with DNA change c.1111G>T.

acute respiratory syndrome (SARS) coronavirus infection was observed for Beijing and Guangzhou populations [21].

Multivariate analysis, including that for IFN response and liver fibrosis, did not reveal an association with MASP-2 levels or the polymorphisms studied. These results suggest that MASP-2 *per se* does not play an immunomodulatory role during treatment with IFN and ribavirin. It has been shown that MBL binds to glycoprotein (E1/E2) of HCV particles, activating MASP-2, which results in neutralization of this virus [22]. If MASP-2 is nonfunctional, the complement cascade can be interrupted and can contribute to viral persistence.

Several studies have indicated an association of *MBL2* with hepatitis C [23,24,25]. Alves Pedroso et al. (2008) [14], studying the *MBL2* gene polymorphism of the promoter region and exon 1 in patients with chronic disease, reported that some genotypes (XA/XA, XA/YO and YO/YO) were associated with lower levels of MBL in patients and that *MBL2* polymorphisms may therefore be associated with development of chronic hepatitis C. Halla et al. (2010) [25], investigating the *MBL2* gene in 186 Brazilian HCV patients, suggested that the HYO haplotype could be associated with HCV infection and liver fibrosis.

In the present study, analyzing previous data from our group [14], the following *MBL2* genotypes were observed in patients homozygous for the G allele of the *MASP2* p.D371Y variant (c.1111G > T); LYPB/LXPA, LYQA/HYPA, HYPD/LYPA, and HYPA/HYPA. Further investigation is necessary to relate the effect of *MASP2* gene polymorphisms and specific *MBL2* genotypes in HCV patients.

In conclusion, *MASP2* polymorphisms, particularly the p.D371Y variant, may increase the risk of developing chronic hepatitis C. These data suggest that genetic factors, in addition to environmental factors, are important in the development and progression of HCV infection. These results, however, should be replicated in different populations to confirm the genetic influence of this SNP in hepatitis C.

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Appendix. Supplementary data

Supplementary data associated with this article can be found, in the online version, at 10.1016/j.humimm.2011.06.016.

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