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Lack of association between genetic variants in the mannose-binding lectin 2 (MBL2) gene and HPV infection

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Abstract. Genetic variants in the immunomodulatory gene mannose-binding lectin 2 (MBL2), were associated with risk, severity, and frequency of viral infections. In a case-control setting, we investigated the association of MBL2 functional polymorphisms with Human Papillomas Virus (HPV) infection. No differences between cases (HPV^+) and controls (HPV^-) were found in the distribution of each single genotypes or allele. Haplotype analysis did not show any difference between HPV+ and HPV^- groups.

Key words: Genetic variants, Gynecological cancer/cervical, Host–virus interaction, HPV, MBL2

Abbreviation: MBL2 = Mannose-Binding Lectin 2; MBP = Mannose-Binding Protein; HPV = Human Papillomas Virus; CIN = Cervical Intraepithelial Neoplasia; RFLP = Restriction Fragment Length Polymorphisms; SNP = Single Nucleotide Polymorphism; GLM = General Regression Model; HW = Hardy-Weinberg; HIV = Human Immunodeficiency Virus; HBV = human Hepatitis B Virus; HCV = Human Hepatitis C Virus

Infection with oncogenic Human Papillomas Virus (HPV) is strongly associated to the development of squamous cell carcinoma of the uterine cervix, the second most frequent cancer in women. Although HPV infection is a very common sexually transmitted disease the majority of the individuals eliminate the virus without showing any clinical manifestation, with only a few HPV infected subjects that develop cervical cancer [1]. In healthy individuals, HPV infections regress spontaneously in 90% of the cases within 12–36 months. HPV persistence, however, is seen in immunodeficient women, suggesting that factors affecting immune system such as genetic predisposition can potentially influence the ability to clear an HPV infection [1, 2]. The protein encoded by MBL2 gene, the mannose-binding lectin (MBL) or mannose-binding protein (MBP), is a recognition molecule that plays a central role in innate immunodefence [3]. Genetic variants of human MBL2 coding sequence have been associated with increased risk of viral infections [4–10]. Reduced MBL serum concentrations are strongly associated with three genetic variants in the exon 1 of the MBL2 gene. The normal MBL allele is termed A. The B variant has a G → A mutation in codon 54 which results in Gly → Asp substitutions, whereas the C variant shows a G → A mutation in codon 57 which leads to a Gly → Asp substitution. The third variant, MBL-D, is a C → T mutation in codon 52 resulting in Arg → Cys substitutions. In addition to these functional variants, two further polymorphisms in the promoter region of the MBL2 gene have been associated to ethnical differences in MBL serum levels. These polymorphic sites are localized at nucleotides –550 and –221, and both shows a single G → C nucleotide polymorphism [3].

In an effort to better understand the mechanisms involved in HPV infection, and thus cervical cancer, we evaluated in a Caucasian case-control setting five polymorphisms in the MBL2 gene as possible risk

Table 1. MBL2 genotype and estimated allele frequencies

	HPV^+ (n = 165)	HPV^- (n = 195)	p	OR	95% CI
Promoter region					
H/L ($G^{-550} \rightarrow$ C)					
G/G	17.20%	20.0%	0.587	1.205	(0.704–2.063)
G/C	66.20%	68.20%	0.735	1.092	(0.701–1.701)
C/C	16.60%	11.80%	0.222	0.674	(0.370–1.227)
G	0.503	0.541	0.329	1.164	(0.867–1.563)
C	0.497	0.459		0.859	(0.640–1.153)
Y/X ($G^{-221} \rightarrow$ C)					
G/G	62.0%	69.70%	0.145	1.415	(0.912–2.196)
G/C	30.60%	27.20%	0.484	0.844	(0.533–1.334)
C/C	7.4%	3.1%	0.088	0.399	(0.147–1.089)
G	0.773	0.833	0.046	1.468	(1.013–2.129)
C	0.227	0.167		0.681	(0.470–0.988)
Coding region (exon 1)					
rs5030737 ($Arg^{52} \rightarrow$ Cys)					
C/C	98.20%	94.80%	0.154	0.347	(0.094–1.282)
C/T	1.20%	2.10%	0.692	1.686	(0.305–9.324)
T/T	0.60%	3.10%	0.132	5.143	(0.613–43.163)
C	0.988	0.959	0.022	0.290	(0.096–0.877)
T	0.120	0.410		3.444	(1.140–10.405)
rs1800450 ($Gly^{54} \rightarrow$ Asp)					
G/G	71.10%	64.10%	0.175	0.724	(0.462–1.132)
G/A	26.40%	32.30%	0.246	1.332	(0.841–2.109)
A/A	2.50%	3.60%	0.760	1.480	(0.426–5.148)
G	0.844	0.803	0.171	0.754	(0.511–1.113)
A	0.156	0.197		1.327	(0.899–1.958)
rs1800451 ($Gly^{57} \rightarrow$ Asp)					
G/G	96.30%	92.30%	0.119	0.459	(0.174–1.211)
G/A	3.70%	7.70%	0.119	2.181	(0.826–5.756)
A/A	–	–	–	–	–
G	0.982	0.962	0.125	0.469	(0.180–1.222)
A	0.180	0.380		2.133	(0.818–5.563)

Abbreviations: HPV^+, HPV positive; HPV^-, HPV negative; OR, odds ratio; 95% CI, 95% of confidence interval.

factors for HPV infection. The study includes 163 unrelated HPV^+ female (cases) and 195 unrelated HPV^- female (controls) both recruited from center-southern Italy. Mean age for the HPV^+ was 36.98 ± 9.66; mean age for the HPV^- was 31.64 ± 5.32. Both cases and controls underwent an identical interview with a standard questionnaire on gynecological history, lifestyle, history of cancer in relatives, menstrual and reproductive factors and use of contraceptives and hormone therapies. Seventy-five of the HPV^+ females (46%) reported at least one pregnancy and 33% at least one live birth. Miscarriage was reported in 10% of the cases and abortion in 24%. Of the 163 cases, 50 (31%) showed concomitant cervical neoplasia. In 21 cases (13%) histopathological examination demonstrated CIN1 lesions, in 14 cases CIN2 lesion (9%), in 10 cases (6%) CIN3 lesions were detected, and the remaining 5 cases (3%) showed invasive squamous cervical carcinomas. Sixty percent of the HPV^+ female hold a high school degree, 25% a university degree and the remaining a middle school (13%) or primary school (2%) degree. Thirty-five percent (n = 55) of the cases were employees, 25% housewives (n = 42), 15% professional (n = 25), 13% students (n = 22), and 12% workers (n = 19). Forty-seven percent of the cases (n = 77) reported more than four sexual partners. Controls were recruited among age controlled healthy women without evidence of HPV infection. No differences were founded in the demographic characteristics between HPV^+ and HPV^- groups. Genotyping was performed by restriction enzyme analysis. A standard PCR approach was used for polymorphisms rs1800451 and Y/X ($G^{-221} \rightarrow$ C), both recognized by MboII restriction enzymes. For the analysis of polymorphisms H/L ($G^{-550} \rightarrow$ C) and

Table 2. Estimated haplotype frequencies in the promoter and in the coding region of MBL2 gene

Haploype	HPV^+	HPV^-	OR	95% CI
Y/X - rs1800450 - rs1800451				
GGG	0.60377	0.60900	1.039	(0.679–1.589)
CGG	0.22138	0.16667	0.719	(0.425–1.216)
GGA	0.15083	0.18588	1.250	(0.715–2.186)
GAG	0.01840	0.02690	1.404	(0.330–5.964)
CGA	0.00561	–	–	–
GAA	–	0.01156	–	–

Abbreviations: HPV^+, HPV positive; HPV^-, HPV negative; OR, odds ratio; 95% CI, 95% confidence interval. Linkage disequilibrium estimates: Y/X-rs1800450, $p = 0.476$ (HPV^+) and $p = 0.265$ (HPV^-); Y/X-rs1800451, $p = 0.002$ (HPV^+) and $p < 0.001$ (HPV^-); rs1800450 - rs1800451, $p = 0.187$ (HPV^+) and $p = 0.534$ (HPV^-).

rs5030737 ($C^{154} \rightarrow T$) a PCR site direct mutagenesis was used. For the H/L polymorphisms, in presence of the C allele at position –550, PCR primers creates a *Drd*I restriction site, whereas in polymorphism rs5030737, in presence of the T allele, a base substitution in the reverse primer ($A \rightarrow T$) introduces an *Hph*I restriction site. The SNP rs1800451 ($G^{170} \rightarrow A$) was analyzed as previously described [6]. Genotype frequencies were compared by means of univariate GLM (General Regression Model) using Bonferroni post-hoc test for multiple comparisons. The agreements of the observed genotype frequencies with the expected Hardy-Weinberg (HW) frequencies were verified with the Arlequin Software, version 2.000. Relative allele frequencies were estimated by the gene counting method, and were compared by means of Fisher's exact test. Fisher's exact test was also used to compare haplotype frequencies through the study groups. Two-tailed *p* value, odds ratios (OR) and the 95% of confidence intervals (CI) were calculated. Arlequin Software, version 2.000 was also used to estimate haplotype frequencies and significance of linkage disequilibrium for each pair of marker through the study groups. Linkage disequilibrium coefficents (D) were estimated as already described [11]. Level of statistical significance was set at $p < 0.05$. All statistical analyses were performed using the SPSS version 10.1.3 statistical software package (SPSS Inc, Chicago, IL, USA).

The allele frequencies in HPV^+ and HPV^- groups were in Hardy-Weinberg equilibrium, except for rs5030377 ($p = 0.033$ and $p = 0.005$, respectively) and for the H/L polimorphism ($p < 0.001$ and $p < 0.001$, respectively). The frequencies and distribution of genotypes and alleles, and the odds ratios for the comparison of each genotype/allele between cases and controls are shown in Table 1. When HPV^+ and HPV^- populations were compared, no differences were found in either single genotypes or allele distribution for all five polymorphisms. Haplotype frequencies in HPV^+ and HPV^- populations are shown in Table 2, as well as the ORs for the comparison of each haplotype between HPV^+ and HPV^- groups. In both cases and controls, because of the significant differences in respect to the HW frequencies, the H/L polymorphism (promoter region) and the rs5030737 polymorphism (coding region) were excluded from the analysis. Haplotype G-G-G is the most frequent, representing from 60.38% (HPV^+) to 60.90% (HPV^-) of the total haplotype frequencies, followed by haplotype C-G-G (22.14% and 16.67%, respectively) and G-G-A (15.08% and 18.8%, respectively). No differences were found when the frequency of the four most frequent expected haplotypes were compared between HPV^+ and HPV^- groups ($p = 0.278$). Despite the exclusion of the H/L and rs5030737 polymorphisms, we were able to identify the major MBL2 alleles, as described in the literature [3]. Haplotype –GG (A + D alleles) is the most common in both HPV^+ and HPV^- groups, representing about 82.5% (HPV^+) and 77.6% (HPV^-) of the total haplotype frequency, followed by the –GA haplotype (C allele), and –AG haplotype (B allele). No differences were found in haplotype distribution when HPV^+ and HPV^- groups were compared. Interestingly, the minor C–G–A haplotype was not present in the HPV^- group, whereas the minor G–A–A haplotype was not present in the HPV^+ group.

An effective immunodefence against pathogens results from the interplay of non-specific innate immunity and antigen specific adaptative immunity. The known mechanisms of MBL2 immunomodulation is the activation of the complement cascade. Genetic polymorphisms in promoter and coding regions of the MBL2 gene, however, have been shown to modulate immune response to viral diseases such as HIV, HCV and HBV infection [4, 7, 10]. This might be due to the recognition of surface molecules on the viral envelope with subsequent complement activation or to an unknown alternative immunomodulatory mechanism [7]. We found no association between MBL2 genotypes and HPV infection, although the possibility of a protective effect the G–A–A haplotype and a risk effect of the C-A-G haplotype cannot be excluded.

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