International Journal of Infectious Diseases 19 (2014) 100–102

Contents lists available at ScienceDirect

International Journal of Infectious Diseases

journal homepage: www.elsevier.com/locate/ijid

Short Communication

Mannose-binding lectin genetic analysis: possible protective role of the HYPA haplotype in the development of recurrent urinary tract infections in men

Olga Scudiero [a,b,*], Maria Ludovica Maurizio Guida [d], Attilio Di Spiezi Giuseppe Castaldo [a,b]

[a] Dipartimento di Medicina Molecolare e Biotecnologie Mediche, Università di Napoli Federico II, Napoli, Italy
[b] CEINGE–Biotecnologie Avanzate, Napoli, Italy
[c] IRCCS–Fondazione SDN, Napoli, Italy
[d] Dipartimento di Medicina e Chirurgia, Catterdra di Ginecologia e Ostetricia, Università degli Studi di Salerno, Salerno, Italy
[e] Dipartimento di Scienze Ostetriche e Ginecologiche, Università degli Studi di Napoli Federico II, Napoli, Italy
[f] Dipartimento di Neuroscienze, Scienze Riproduttive e Odontostomatologiche, Università degli Studi di Napoli Federico II, Napoli, Italy
[g] Dipartimento di Scienze e Tecnologie Ambientali Biologiche Farmaceutiche, Seconda Università degli Studi di Napoli, Caserta, Italy

ARTICLE INFO

Article history:
Received 16 September 2013
Received in revised form 25 October 2013
Accepted 28 October 2013

Corresponding Editor: Eskild Petersen, Aarhus, Denmark

Keywords:
MBL
rUTI
Molecular analysis
Haplotype

SUMMARY

Factors related to bacterial virulence and/or to the host have been implicated in the pathogenesis of recurrent urinary tract infections (rUTI), but in most cases the cause is unknown. Mannose binding lectin (MBL) is an acute phase glycoprotein that exerts immunological functions by binding to the surface of a variety of pathogens. Some human gene variants reduce MBL activity thereby predisposing the host to bacterial and viral infections. The aim of this study was to investigate MBL2 gene variants in relation to rUTI risk. Six MBL gene variants and seven haplotypes were analyzed by PCR and direct sequencing in rUTI patients ($n = 83$) and in healthy subjects from southern Italy ($n = 642$). The frequencies of the L allele (−550) and the HYPA haplotype were higher in controls than in patients stratified according to sex ($p < 0.05$). Our data indicate that the HYPA haplotype in the MBL2 gene could be associated with a minor risk of developing rUTI in males.

1. Introduction

Recurrent urinary tract infections (rUTI) are symptomatic episodes that follow the resolution of earlier episodes.[1] rUTI due to chronic bacterial infections is less frequent in men than in women.[1,2] Several factors related to bacteria or to the host, including genetic susceptibility,[3] have been implicated in rUTI pathogenesis. Mannose binding lectin (MBL), which is an acute phase serum glycoprotein that exerts immunological effects,[4,5] may play a role in the pathogenesis of rUTI.

Common MBL nucleotide alterations are essentially located in the promoter region and/or in the coding region of the MBL2 gene. They reduce the synthesis and/or the function of the gene thereby resulting in increased susceptibility to bacterial infections.[4,5] MBL activity has been associated with various diseases.[6,7] MBL2 is a modifier gene for the cystic fibrosis phenotype, and alterations in this gene have been associated with gastric cancer in patients with *Helicobacter pylori* infection.[6,7] To date, no data have been reported regarding the relationships between MBL2 gene variants and susceptibility to rUTI.

To investigate whether MBL variants are involved in rUTI pathogenesis, we studied the structural and promoter alleles, genotypes, and haplotypes of the MBL2 gene in a cohort of rUTI patients compared to a cohort of healthy subjects living in southern Italy.

2. Materials and methods

Forty-six unrelated men with rUTI (25–80 years, mean 59.9 years) and 37 women with rUTI (21–49 years, mean 39 years)[8] were enrolled in the study. None had a history of surgery, and none

* Corresponding author. Tel.: +39 081 3737819; fax: +39 081 3737808.
E-mail address: olga.scudiero@unina.it (O. Scudiero).

1201-9712
http://dx.doi.org/10.1016/j.ijid.2013.10.019

Table 1
Genotype frequencies in patients and controls

Position and allelic genotype			Control F (n = 308), n (%)	Control M (n = 334), n (%)	Case F (n = 37), n (%)	Case M (n = 46), n (%)	[a]p-Value	[b]p-Value	OR (95% CI)	OR (95% CI)
Promoter	Position −550	H/H	103 (33.4)	111 (33.2)	15 (40.5)	23 (50.0)	0.69	**0.02**	0.90 (0.55–1.48)	**0.55 (0.33–0.90)**
		H/L	162 (52.6)	178 (53.3)	16 (43.2)	21 (45.7)				
		L/L	43 (14.0)	45 (13.5)	6 (16.2)	2 (4.3)				
	Position −221	Y/Y	187 (60.7)	200 (59.9)	23 (62.2)	27 (58.7)	0.88	0.99	0.95 (0.53–1.71)	1.00 (0.59–1.68)
		Y/X	104 (33.8)	116 (34.7)	12 (32.4)	17 (37.0)				
		XX	17 (5.5)	18 (5.4)	2 (5.4)	2 (4.3)				
	Position +4	P/P	199 (64.6)	217 (65.0)	19 (51.4)	25 (54.3)	0.15	0.08	1.50 (0.86–2.59)	1.55 (0.94–2.56)
		P/Q	96 (31.2)	105 (31.4)	16 (43.2)	17 (37.0)				
		Q/Q	13 (4.2)	12 (3.6)	2 (5.4)	4 (8.7)				
Exon 1										
Codon 52	Position +223	A/A	275 (89.3)	305 (91.3)	34 (91.9)	41 (89.1)	0.79	0.58	0.74 (0.22–2.49)	1.35 (0.50–3.61)
		A/D	33 (10.7)	27 (8.1)	3 (8.1)	5 (10.9)				
		D/D	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)				
Codon 54	Position +230	A/A	238 (77.3)	257 (76.9)	31 (83.8)	29 (63.0)	0.24	0.08	0.60 (0.25–1.42)	1.64 (0.93–2.89)
		A/B	61 (19.8)	68 (20.4)	6 (16.2)	16 (34.8)				
		B/B	9 (2.9)	9 (2.7)	0 (0.0)	1 (2.2)				
Codon 57	Position +239	A/A	300 (97.4)	322 (96.4)	35 (94.6)	45 (97.8)	0.64	0.6	2.11 (0.44–10.13)	0.60 (0.07–4.67)
		A/C	8 (2.6)	12 (3.6)	2 (5.4)	1 (2.2)				
		C/C	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)				

F, females; M, males.
[a] p-Values from allele comparison of case F vs. control F.
[b] p-Values from allele comparison of case M vs. control M.

had anatomical abnormalities. The inclusion criterion for men was a positive Meares test.[9] The etiological agents identified in the 46 male patients were *Escherichia coli* (69.5%), *Proteus mirabilis* (13%), *Klebsiella pneumoniae* (8.6%), and *Streptococcus faecalis* (4.3%). Two patients (4.3%) had Gram-positive Corynebacterium. The inclusion criteria for women were: not pregnant or pre-menopausal, no functional or structural abnormalities, and the occurrence of two rUTIs in 6 months or three episodes per year. The bacteria identified in the 37 women were *E. coli* (88%), *S. faecalis* (5%), *P. mirabilis* (2.5%), *K. pneumoniae* (2.5%), and *Pseudomonas spp* (2%). Laboratory analyses of the entire cohort of patients screened excluded leukocyte, immunoglobulin, and complement alterations. The control group comprised 642 unrelated healthy adults from southern Italy (334 men and 308 women).

All individuals enrolled in the study provided written informed consent. The study was approved and conducted in accordance with the ethical principles stated in the most recent version of the Declaration of Helsinki.

Genomic DNA was extracted from each blood sample using commercial procedures. DNA was analyzed for the MBL2 gene (the promoter, 5′ untranslated region (UTR), and exon 1) by PCR followed by direct sequencing. MBL2 mutations in exon 1 at codons 52, 54, and 57 are defined D, B, and C, respectively. The wild-type allele is 'allele A'. The promoter polymorphisms are H/L (−550), Y/X (−221), and P/Q (+4 in the 5′ UTR). Seven MBL2 haplotypes were obtained from the combination of the six gene variants.[6,7,10]

Hardy–Weinberg equilibrium was evaluated with the goodness-of-fit Chi-square test. Differences in the distribution of allele frequencies between patients and controls were evaluated with a two-sided Chi-square test. Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated to assess the relative risk conferred by a specific allele. Statistical significance was established at $p \leq 0.05$.

3. Results

The genotype and haplotype frequencies of single nucleotide polymorphisms (SNPs) in the MBL2 gene of rUTI patients and healthy controls stratified by gender are summarized in Tables 1 and 2. At position −550 of the promoter, the L allele was significantly more frequent in male controls than in patients (p = 0.02). Therefore, we evaluated the MBL2 haplotypes resulting from the combination of the six gene variants and identified seven previously reported haplotypes. Statistical analysis without correcting for multiple tests revealed that the HYPA (wild-type) haplotype shows a trend towards an association with a lower risk of developing rUTI infections in males (Table 2, p = 0.02).

Table 2
Comparison of haplotype frequencies between healthy controls and rUTI patients

Haplotype	Controls F (n = 616), n (%)	Controls M (n = 668), n (%)	Cases F (n = 74), n (%)	Cases M (n = 92), n (%)	[a]p-Value	[b]p-Value
HYPA	235 (39)	253 (38)	28 (38)	24 (26)	0.87	**0.02**
HYPB	16 (0.9)	17 (2.5)	0 (0)	1 (1.1)	0.32	0.61
LXPA	104 (17)	120 (18)	10 (13.5)	14 (15.2)	0.43	0.51
LXPB	4 (0.6)	4 (0.6)	0 (0)	1 (1.1)	0.92	0.88
LXQA	30 (5)	28 (4.2)	6 (8)	6 (6.6)	0.25	0.31
LYPA	127 (21)	137 (20.5)	15 (20)	25 (27.2)	0.89	0.14
LYPB	8 (1)	8 (1.2)	2 (2.7)	2 (2.2)	0.67	0.77
LYQA	87 (14.3)	99 (14.5)	13 (17.6)	18 (19.6)	0.46	0.23
LYQB	5 (0.8)	2 (0.3)	0 (0)	1 (1.1)	0.95	0.8

F, females; M, males.
[a] p-Values from allele comparison of case F vs. control F.
[b] p-Values from allele comparison of case M vs. control M.

4. Discussion

A deficiency of the MBL protein is correlated with an increased susceptibility to various infectious states.[5] Mutations in the promoter region of the MBL gene (i.e., −550C, −221C, and +4T) cause reduced protein synthesis, while alterations in exon 1 (i.e., R52C, G54D, and G57E) give rise to peptides less able to polymerize, thereby resulting in a lower affinity for the bacterial surface[4] and reduced pro-complementary activity.[4,5] Moreover, altered MBL2 haplotypes decrease the activities of the immunological protein and increase the occurrence of infections.[5]

In this study of rUTI patients, we found that the frequencies of the L allele and of the wild-type HYPA haplotype differed significantly between healthy subjects and rUTI males. To date, there is no evidence that the L allele in heterozygosis affects immunological protein activity. Reduced synthesis of MBL occurs when at least two promoter mutations are present in heterozygosis, which indicates that a single polymorphism probably does not significantly affect the rUTI risk.[10] On the other hand, considering the entire haplotype, we found a significantly higher frequency of the HYPA haplotype in control males than in rUTI males. HYPA is among the most frequent haplotypes in the normal population and correlates with high MBL levels. Our data, although preliminary, suggest that the HYPA MBL2 haplotype may act as a protective factor against rUTI in males.

In conclusion, this is the first study to investigate the correlation between MBL2 gene variants and rUTI. Our results indicate that molecular analysis (easily performed by PCR on blood genomic DNA) could be a diagnostic tool with which to identify subjects predisposed to rUTI.

Acknowledgements

Grants from Regione Campania (L. 502/92, annualità 2004, 2005 and contract for molecular diagnostics, DGRC n 1901 del 22/12/2009) are gratefully acknowledged. We thank Jean Ann Gilder (Scientific Communication srl, Naples, Italy) for editing the text.

Funding: The sponsors had no involvement.

Conflict of interest: The authors have no conflict of interest.

References

1. Mak RH, Kuo HJ. Pathogenesis of urinary tract infection: an update. *Curr Opin Pediatr* 2006;**18**:148–52.
2. Colodner R, Nitzan O, Chazan B, Edelstein H, Raz R. Mannose-binding lectin in pre-menopausal women with recurrent urinary tract infections. *Clin Microbiol Infect* 2010;**16**:1394–8.
3. Kilpatrick DC. Mannan binding lectin and its role in innate immunity. *Transfus Med* 2002;**12**:335–52.
4. Tomaiuolo R, Ruocco A, Salapete C, Carru C, Baggio G, Franceschi C, et al. Activity of mannose-binding lectin in centenarians. *Aging Cell* 2012;**11**(3):394–400.
5. Thiel S, Frederiksen PD, Jensenius JC. Clinical manifestations of mannan-binding lectin deficiency. *Mol Immunol* 2006;**43**:86–96.
6. Scudiero O, Nardone G, Omodei D, Tatangelo F, Vitale DF, Salvatore F, et al. A mannose-binding lectin-defective haplotype is a risk factor for gastric cancer. *Clin Chem* 2006;**52**:1625–7.
7. Tomaiuolo R, Degiorgio D, Coviello DA, Baccarelli A, Elce A, Raia V, et al. An MBL2 haplotype and ABCB4 variants modulate the risk of liver disease in cystic fibrosis patients: a multicentre study. *Dig Liver Dis* 2009;**41**:817–22. http://dx.doi.org/10.1016/j.dld.2009.03.012.
8. Nyberg LM, Krieger JN, Nickel JC. National Institutes of Health Classification of chronic prostatitis. In: Nickel JC, editor. *Textbook of prostatitis*. Oxford, UK: Isis Medical Media; 1999. p. 27–9.
9. Meares Jr EM. Bacterial prostatitis vs prostatosis: clinical and bacteriological study. *JAMA* 1973;**224**:1372–5.
10. Wallis R, Cheng JY. Molecular defect in variant forms of mannose-binding protein associated with immunodeficiency. *J Immunol* 1999;**163**:4953–9.