

# The mannose binding lectin gene influences the severity of chronic liver disease in cystic fibrosis

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## Abstract

**Chronic liver disease is a major complication of cystic fibrosis. Its incidence and severity show marked heterogeneity, even among the homogeneous group of homozygous  $\Delta F508$  patients, suggesting that environmental or genetic factors other than the deletion  $\Delta F508$  may influence the development of cystic fibrosis related liver disease. We investigated whether the allelic variants of mannose binding lectin, an important protein of the immune system, could be associated with the presence of cirrhosis in a population of 216 homogeneous homozygous  $\Delta F508$  patients. Analysis of the data shows that the presence of cirrhosis in cystic fibrosis patients is significantly associated with a mutated mannose binding lectin genotype (homozygous or compound heterozygous for mannose binding lectin variants). The modulating role of mannose binding lectin in the occurrence of cirrhosis in cystic fibrosis could be explained by the fact that hepatotoxic damage from viruses or bacteria might be increased by the immunodeficiency associated with mannose binding lectin variants and might facilitate the degradation of liver status. These data highlight the crucial role of mannose binding lectin in the clinical outcome of cystic fibrosis, as it has recently been shown that the mannose binding lectin gene is a modulating gene of the respiratory involvement in cystic fibrosis patients.**

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Chronic liver disease is a major complication of cystic fibrosis, which may lead to cirrhosis and limit the survival and quality of life of affected patients. Its pathogenesis is incompletely explained by the effects of defective cystic fibrosis transmembrane regulator on the secretory function of the biliary epithelium.<sup>1</sup> Cystic fibrosis associated chronic liver disease shows a substantial variability both in terms of incidence and severity, even among  $\Delta F508$  homozygous patients.<sup>2</sup> This could be explained by the influence of environmental factors, such as hepatotoxic damage from viruses, bacteria, and drugs,<sup>3</sup> or genetic factors other than the cystic fibrosis transmembrane regulator gene,<sup>4</sup> which may increase susceptibility to hepatotoxic

factors. The influence of the major histocompatibility complex has thus been recently described.<sup>5</sup>

We investigated the possible modulating role of the mannose binding lectin, an important protein of the innate immune system synthesised by the liver, in the occurrence of cirrhosis in cystic fibrosis patients, because (1) heterozygosity or compound heterozygosity for mannose binding lectin variant alleles is responsible for a decrease in the protein plasma concentration, an opsonic defect, and a common immunodeficiency,<sup>6,7</sup> and (2) these variants are suspected to be associated with an adverse outcome of hepatitis B and C virus infection.<sup>8,9</sup>

## Material and methods

We studied the association between the allelic variants of mannose binding lectin and the presence of liver cirrhosis among cystic fibrosis patients. To avoid any phenotypic heterogeneity as a result of allelic heterogeneity in the cystic fibrosis transmembrane regulator gene, we identified, in association with the French National Observatory for Cystic Fibrosis, a homogeneous homozygous  $\Delta F508$  population of 216 patients, whose mannose binding lectin genotype was determined by means of denaturing gradient gel electrophoresis.<sup>10</sup> The presence of liver cirrhosis was defined on the basis of the association of firm hepatomegaly, ultrasonographic findings of liver heterogeneity and micro- or macronodular formation, and portal hypertension, defined as the presence of splenomegaly, reversed blood flow in the portal vein, and dilated collateral veins.

## Results

Among the 216 patients, we identified two groups: (1) a first group of 203 patients homozygous or heterozygous for mannose binding lectin wild type alleles (NN+NM group), of whom 11 (5.4%) showed evidence of cirrhosis, and (2) a second group of 13 patients homozygous or compound heterozygous for mutations in the mannose binding lectin gene (MM group), of whom four (30.8%) had cirrhosis. The two groups were comparable in age and sex ratio (mean age 14.5 (SD 8.7) and 17.9 (SD 8.4) years respectively, *t* test; sex ratio 0.86 and 1.43 respectively, corrected chi-square). Analysis of the data showed a significantly higher prevalence of cystic fibrosis related cirrhosis in the group of patients homozygous or compound heterozygous for mutations in the mannose binding lectin gene (MM group) compared with the group of patients homozygous or heterozygous for its wild type

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Table 1 Liver status split by mannose binding lectin genotype in 216  $\delta F508$  homozygous patients

MBL genotype	Cirrhosis	Non-cirrhosis	% Cirrhosis	p values (Fisher's exact test)
Wild type (NN) n=131 (60.7%)	n=9	n=122	6.9%	
Heterozygotes (NM) n=72 (33.3%)	n=2	n=70	2.8%	NS*
52/N n=18	1	17		
54/N n=46	1	45		
57/N n=8	0	8		
Subtotal (NN+NM) n=203 (94.0%)	n=11	n=192	5.4%	0.008† [OR: 7.76 95% CI: 2.01–29.00]
Mutated homozygotes or compound heterozygotes (MM) n=13 (6.0%)	n=4	n=9	30.8%	0.045* (OR 6.02, 95% CI: 1.51–24.10)
54/54 n=5	2	3		
57/57 n=1	0	1		
52/54 n=6	2	4		
54/57 n=1	0	1		
Total n=216 (100%)	n=15	n=201	6.9%	

MBL: mannose binding lectin. NS: not significant. OR: odds ratio.

\*Compared to NN group.

†Compared to MM group.

The mannose binding lectin genotype frequencies are in Hardy-Weinberg proportions for all the 216 patients. Sex ratio and mean age of the cirrhotic and the non-cirrhotic groups do not differ statistically. The frequency of the MBL mutated alleles is 0.33 and 0.22 in the cirrhotic and non-cirrhotic groups, respectively. This difference is not significant because the frequency of cirrhosis in the NM group is low, but not statistically different from that observed in the NN group. Nearly half of the population comes from adult centres.

allele (NN+NM group) ( $p=0.008$ ) and with the group of patients homozygous for its wild type allele (MM group) ( $p=0.045$ ) (table 1).

### Discussion

This study shows that an independent genetic factor, the mannose binding lectin gene, influences liver disease severity in cystic fibrosis  $\Delta F508$  homozygous patients, mannose binding lectin variants being associated with an increased risk of developing cirrhosis. This could be explained by the fact that the immunodeficiency associated with mannose binding lectin variants might increase the hepatotoxic damage from infectious agents and therefore facilitate the degradation of the liver status in cystic fibrosis patients. These new data suggest that mannose binding lectin, which has also been reported by us<sup>11</sup> and others<sup>12</sup> as acting as a modulating gene of the respiratory involvement in cystic fibrosis patients, plays a crucial role in the clinical outcome of these patients.

This suggests the need for screening such patients for the mannose binding lectin gene, in order to identify at risk patients, who could benefit from adapted follow up and clinical care, as the effectiveness of bile acid therapy might be higher if started in patients with early stage liver disease.<sup>13</sup>

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