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## Increased risk of NASH in patients carrying the C(−159)T polymorphism in the CD14 gene promoter region

Non-alcoholic fatty liver (NAFL) is a common hepatic disorder that progresses to non-alcoholic steatohepatitis (NASH) in only 20% of patients. Whereas polymorphisms in genes involved in fat metabolism confer susceptibility to NAFL,[1] the risk factors involved in the progression of the disease to NASH are not known. A possible role for intestinal derived bacterial endotoxins in the progression from NAFL to NASH is gaining increasing interest in view of recent experimental data. Thus NASH patients show a higher prevalence of small intestinal bacterial overgrowth (SIBO) and, in animal models of SIBO, hepatitis is improved following antibiotic treatment.[2 3] Furthermore, we have recently observed that increased intestinal mucosal permeability, such as that observed in obese C57BL/6J$^{ob/ob}$ mice, leads to higher lipopolysaccharide (LPS) levels in portal blood (P Brun, manuscript submitted). Circulating LPS binds to soluble and cell membrane receptors, such as CD14 and toll-like receptor 4 (TLR4), leading to release of inflammatory cytokines involved in the pathogenesis of NASH such as tumour necrosis factor α (TNF-α), interleukin (IL)-1β, and IL-6. Nevertheless, recent studies demonstrate that genetic polymorphisms in CD14 and TLR4 genes greatly influence the amplitude of individual inflammatory response to LPS.[4 5]

These observations prompted us to investigate whether variants in genes coding LPS receptors are associated with a greater risk in progression from NAFL to NASH. To achieve this goal, we performed polymerase chain reaction to amplify the promoter of the CD14 gene (between −408 and −75 bp from the transcription start site) and the fourth exon of the TLR4 gene (between 827 and 1253 bp) on genomic DNA extracted from white blood cells of overweight patients (body mass index 22–27) showing biopsy proven NASH (n = 21) or NAFL (n = 7). Subjects with normal liver function tests randomly recruited among blood donors of the University Hospital of Padua (n = 52) served as the control group. Genetic variants were subsequently determined in both DNA strands using 3100 Analyser Automatic DNA Sequencer (Applied Biosystems, Foster City, California, USA). TNF-α was assessed in the sera of patients and controls using a commercially available ELISA kit (BioSource International, California, USA). Statistical analysis was performed using SPSS.

TT genotype distribution was significantly higher in NASH patients than in control subjects (57.1% *v* 23.1%; odds ratio 3.75) while no TT genotype was observed among the seven NAFL patients (table 1). No difference in the distribution of genetic variants in the TLR4 gene was found between healthy subjects, and NAFL and NASH patients. Circulating levels of TNF-α were significantly ($p<0.05$) higher in NASH than in NAFL patients or controls (15.4 (0.7), 12.9 (0.2), and 9.6 (0.9) pg/ml, respectively). In addition, among NASH patients, subjects carrying the TT genotype had higher TNF-α serum levels than those with the TC and CC genotypes (16.5 (1.2) and 13.6 (0.8) pg/ml, respectively; $p<0.05$), irrespective of body mass index (data not shown).

The functional relevance of the C(−159)T polymorphism in the CD14 gene promoter region of NASH patients stems from the increased CD14 expression associated with the TT genotype leading to enhanced inflammatory responses to circulating endotoxins.[6] Indeed, several hepatic cell populations, including hepatic stellate cells involved in liver damage and fibrogenesis, can directly respond to LPS.[7 8] Thus increased CD14 expression in patients carrying the TT genotype might enhance the sensitivity to intestinal LPS in obese subjects.[9] Moreover, the stronger proinflammatory response will pave the way to progression from NAFL to NASH and cirrhosis. We suggest designing larger and prospective studies to better assess the predisposing role of the C(−159)T polymorphism in the CD14 gene promoter region of NASH patients to identify subjects at risk of developing a more severe liver disease.


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**Table 1** Distribution of C(−159)T CD14 genotypes in patients with non-alcoholic steatohepatitis (NASH) and non-alcoholic fatty liver (NAFL), and in controls

| CD14 genotype | Controls (n = 52) | NAFL (n = 7) | NASH* (n = 21) |
|---|---|---|---|
| C/C | 15 (28.8%) | 0 | 4 (19.0%) |
| C/T | 25 (48.1%) | 7 (100%) | 5 (23.8%) |
| T/T | 12 (23.1%) | 0 | 12 (57.1%) |

*Genotype frequency (3×3 contingency table): NASH *versus* controls $\chi^2 = 17.211$, $p<0.002$.


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## Lavage of a mucinous cystadenoma of the pancreas with povidone iodate

Mucinous cystadenomas of the pancreas may progress to malignancy. Surgery is the treatment of choice.[1 2]

An 88 year old man presented with postprandial vomiting and intractable gastric pain due to an 8 cm pancreatic cyst compressing and displacing the pyloric antrum (fig 1). His picture included (inter alia) ischaemic cardiopathy and he was on radiotherapy for tonsillar squamous cell carcinoma. Ultrasound, computed tomography (CT), and magnetic resonance imaging revealed a non-communicating lesion resembling a mucinous cystadenoma. Aspiration produced a mucinous liquid with 16 U/l amylase, 66 U/l lipase, 234 ng/ml carcinoembryonic antigen, and 16620 μ/ml Ca 19.9. Recurrence of the cyst and symptoms was found a few weeks later. Surgery was ruled out by an ASA IV anaesthesiological risk. Echoendoscopic drainage