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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)-1 β , 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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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 g/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

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 $\times$ 3 contingency table): NASH versus controls $\chi^2 = 17.211$, $p < 0.002$.

Figure 1 (A) Computed tomography scan showing an 8 cm pancreatic body cyst. (B) Cyst regression at six months after lavage with povidone iodine.

was barred by large vessels between the gastric and cystic walls. CT guided aspiration was performed via an 8.4 Fr pigtail catheter. The contrast medium showed that the cyst was not communicating. It was refilled with povidone iodine and emptied after five minutes. The catheter was removed after one week. CT at six months showed complete regression (fig 1) and the symptoms have not returned.

This is the first description of lavage of a mucinous pancreatic cystadenoma with povidone iodine. This substance damages the epithelia and is effective in the treatment of renal cysts,³⁻⁴ fistulae, lymphoceles after renal transplantation,⁵⁻⁶ and hydatid cysts.⁷ It could prove a valid alternative to ethanol⁸ when surgery is impracticable.

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BRAF mutation and gene methylation frequencies of colorectal tumours with microsatellite instability increase markedly with patient age

The microsatellite instability phenotype (MSI⁺) is observed in approximately 25% of colon cancers and 2% of rectal cancers. MSI⁺ is a hallmark of almost all hereditary non-polyposis colorectal cancers (HNPCC) where it is associated with germline mutations in one of the DNA mismatch repair genes.¹ However, the large majority of MSI⁺ cancers occur as sporadic cases that arise following methylation induced silencing of the *hMLH1* gene promoter. Sporadic MSI⁺ tumours are believed to originate in serrated polyps and display frequent and concurrent methylation of gene promoter regions but low frequencies of *KRAS* and *TP53* mutations.² In contrast, HNPCC associated MSI⁺ tumours originate in conventional adenomas and show frequent *APC* and *KRAS* mutations but infrequent methylation. Another striking difference is that *BRAF* mutations occur in most sporadic MSI⁺ tumours but have never been observed in HNPCC-MSI⁺ tumours.³⁻⁴ While there are clearly major differences between sporadic and familial MSI⁺ tumours, we investigated here whether patient age was also an important factor that could influence MSI⁺ tumour phenotype.

The frequency of the *BRAF* mutation was evaluated in MSI⁺ tumours from a consecutive series of colorectal cancer (CRC) patients aged <60 years (n = 828) who were enrolled in a population based screening programme for HNPCC in the state of Western Australia. A total of 66 MSI⁺ cases (8%) were identified using the BAT26 mononucleotide marker, of which only five (7.6%) contained a *BRAF* mutation. Family cancer history and germline mutation status for all 66 MSI⁺ cases have yet to be determined but preliminary data suggest that less than one third will be HNPCC. For comparison, *BRAF* mutations were also investigated in non-selected MSI⁺ patients aged ≥60 years. Of 45 MSI⁺ cases, 27 (60%) showed a *BRAF* mutation, with a highly significant difference in frequency between young and old MSI⁺ patients ($p < 1 \times 10^{-5}$). These results are almost identical to those of another recent population based study which reported a *BRAF* mutation frequency of 7% in MSI⁺ tumours from

patients aged <55 years and 61% in those aged 55–79 years ($p = 0.0002$).⁵

The frequency of methylation in five gene promoter regions (*hMLH1*, *p16*, *p14*, *TIMP3*, and *MINT2*) was also compared between MSI⁺ tumours from young and old patients derived from a non-selected CRC series. In six MSI⁺ tumours from patients aged <60 years, only 2/30 (7%) of the CpG islands investigated by MethyLight assay were methylated. A much higher frequency (61/100, 61%) was observed in 20 MSI⁺ tumours from patients aged ≥60 years ($p < 1 \times 10^{-5}$). These results concur with an earlier study showing that patients with *hMLH1* methylated MSI⁺ tumours were, on average, 18 years older than those without *hMLH1* methylation.⁶

The above results on the frequencies of *BRAF* mutation and promoter methylation demonstrate the existence of striking age related differences in MSI⁺ phenotype. In view of the very low incidence of HNPCC (0.5–1.5% of all CRC), these differences are likely to involve a much greater proportion of MSI⁺ tumours than simply the rare cases with germline mutations in mismatch repair genes. We estimate that 30–40% of MSI⁺ tumours in population based CRC cohorts belong to a subgroup characterised by the absence of both *BRAF* mutation and promoter methylation.

The clinical significance of these findings relates to the potential prognostic and predictive values of MSI⁺. Current disagreement in the literature concerning the predictive value of MSI⁺ for response to 5-fluorouracil (5FU) chemotherapy⁷⁻¹⁰ could be due to the relative proportion of HNPCC and younger MSI⁺ cases included within these studies. *BRAF* mutation and gene promoter methylation, or other factors closely associated with these features, may be strong determinants of the response to 5FU. We therefore recommend consideration of this issue in future studies aimed at evaluating the predictive significance of MSI⁺ in colon cancer.

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