**Table 1** Baseline histological features and changes after 6 months of treatment with orlistat

| Patient | Fat extension before treatment | Fat extension after treatment | Inflammation before treatment | Inflammation after treatment | Fibrosis before treatment | Fibrosis after treatment | Weight change (%) |
|---|---|---|---|---|---|---|---|
| 1 | Severe | Severe | A2 | A0 | F2 | F0 | −2.9 |
| 2 | Severe | Mild | A2 | A1 | F2 | F1 | −4.5 |
| 3 | Mild | Normal | A4 | A2 | F2 | F3 | −8.4 |
| 4 | Mild | Normal | A2 | A2 | F1 | F1 | −2.4 |
| 5 | Severe | Mild | A2 | A0 | F2 | F0 | −8.2 |
| 6 | Severe | Severe | A2 | A1 | F2 | F1 | −2.3 |
| 7 | Moderate | ild | A1 | A1 | F1 | F0 | −5.5 |
| 8 | Severe | Severe | A2 | A1 | F2 | F1 | +3 |
| 9 | Severe | Mild | A2 | A1 | F1 | F0 | −16.3 |
| 10 | Severe | Moderate | A2 | A2 | F2 | F1 | −3.3 |
| 11 | Severe | Severe | A3 | A2 | F3 | F1 | −7.3 |
| 12 | Mild | Normal | A1 | A0 | F1 | F1 | −6.3 |
| 13 | Moderate | Mild | A1 | A0 | F1 | F0 | −4.1 |
| 14 | Severe | Mild | A2 | A0 | F2 | F2 | −6.9 |

Fat extension: normal = <10%, mild = <30%, moderate = 30–60%, severe = >60%. Fibrosis scale: 0 = none, F1 = portal, F2 = portal +septa, F3 = bridging, F4 = cirrhosis. Portal & periportal inflammatory activity (A): 0 = normal, 1 = mild, 2 = moderate, 3 = severe.

at the end of treatment with orlistat (alanine aminotransferase 84 (10) IU/l vs 43 (5) IU/l, p<0.001; aspartate aminotransferase 72 (11) IU/l vs 32 (4) IU/l, p<0.001; total cholesterol 229 (12) mg/dl vs 194 (13) mg/dl, p<0.001), triglycerides 238 (23) mg/dl vs 163 (10) mg/dl, p<0.001) and LDL 143 (11) mg/dl vs 120 (5) mg/dl, p<0.003). Similarly, insulin resistance index and malondialdehyde (MDA) levels improved significantly after orlistat treatment, whereas HbAIc remained unchanged (homeostatic model assessment index, normal 0.8–5.4; 6.5 (2.5) vs 3.3 (1.2), p<0.05; MDA normal <0.3 nmol/ml; 0.47 (0.03) nmol/ml vs 0.37 (0.02) nmol/ml; p<0.01), and (HbAIc normal 3.8–6.4%, 7.1 (3.1)% vs 7.5 (2.6)%, p>0.05).

The mechanism underlying the effect of orlistat remains unknown. Orlistat reduces the absorption of dietary fat and modulate insulin action by changing not only the amount of fat delivered to the liver, but also by changing the type of fat. Saturated fatty acids increase insulin resistance, whereas unsaturated fat, particularly monounsaturated fat, improves insulin sensitivity.[6] Patients with hypertriglyceridaemia have raised tumour necrosis factor-α, which induces systemic inflammation.[7] Whether the reduced levels of postprandial triglycerides induced by orlistat lowers the inflammatory component associated with NASH remains to be determined. MDA may reflect the process of lipid peroxidation occurring in the liver.[8] However, it could be an expression of an enhanced production of free radicals in the circulation as documented in obese patients and patients with diabetes.[9] In our patients, MDA levels were reduced with orlistat treatment, which may be due to the lowering of plasma low density lipopritein (LDL) levels, which may increase cellular LDL receptors synthesis. This will shorten the time of residence of LDL in the plasma, and thereby reduces its exposure to free radicals and preserves LDL antioxidant content, which make it more resistant to oxidation.

One limitation of our clinical study is absence of a placebo group. Thus, all the changes observed could be nonspecific and do not necessarily reflect any unique property of orlistat. Therefore, trials comparing orlistat-induced weight loss against the effect of similar weight loss induced by dieting or exercise are needed before any claim that orlistat has a specific benefit can be substantiated.

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## References

1 **Bugianesi E**, Leone N, Vanni E, *et al*. Expanding the natural history of nonalcoholic steatohepatitis: from cryptogenic cirrhosis to hepatocellular carcinoma. *Gastroenterology* 2002;**123**:134–40.
2 **Angulo P**. Nonalcoholic fatty liver disease. *N Engl J Med* 2002;**346**:1221–31.
3 **Kushner RF**. Medical management of obesity. *Semin Gastrointest Dis* 2002;**13**:123–32.
4 **Davidson MH**, Hauptman J, DiGirolamo M, *et al*. Weight control and risk factor reduction in obese subjects treated for 2 years with orlistat: a randomized controlled trial. *JAMA* 1999;**281**:235–42.
5 **Zavoral JH**. Treatment with orlistat reduces cardiovascular risk in obese patients. *J Hypertens* 1998;**16**:2013–17.
6 **Grundy SM**, Abate N. Chandalia: Diet composition and the metabolic syndrome: what is the optimal fat intake? *Am J Med* 2002;**113**(Suppl 9B):25S–29S.
7 **Jonkers IJ**, Mohrschladt MF, Westendorp RG, *et al*. Severe hypertriglyceridemia with insulin resistance is associated with systemic inflammation: reversal with bezafibrate therapy in a randomized controlled trial. *Am J Med* 2002;**112**:275–80.
8 **Albano E**, Clot P, Morimoto M, *et al*. Role of cytochrome p450 2E1-dependent formation of hydroxyethyl free radical in the development of liver damage in rats intragastrically fed with ethanol. *Hepatology* 1996;**23**:155–63.
9 **Knekt P**, Reunanen A, Jarvinen R, *et al*. Antioxidants, vitamin intake and coronary mortality in a longitudinal population study. *Am J Epidemiol* 1995;**142**:1269–78.

## Screening for coeliac disease in patients fulfilling the Rome II criteria for irritable bowel syndrome in a secondary care hospital in The Netherlands: a prospective observational study

Irritable bowel syndrome is the most commonly diagnosed gastrointestinal condition and affects a large proportion of the population in the West.[1] Several guidelines suggest that in typical patients with no alarm symptoms or signs, apart from routine laboratory tests, no additional tests are necessary.[2] Coeliac disease, however, may present with symptoms suggestive of irritable bowel disease.[3] Several studies suggested that screening for coeliac disease in patients with symptoms suggestive of irritable bowel disease might be cost-effective.[4–7] We, therefore, started to routinely screen patients with typical irritable bowel disease symptoms for coeliac disease using antiendomysial antibodies.

All patients referred to three doctors at the outpatient gastroenterology department of our hospital between November 2002 and November 2005 for suggestive irritable bowel disease and fulfilling the Rome II criteria were included if routine laboratory tests and an endoscopy of the lower gastrointestinal tract were performed. Furthermore, all patients received standard care including an interview and physical examination. Apart from these tests, the treating physician could order any other diagnostic test as considered necessary.

In addition, serological screening for coeliac disease was carried out in all patients. Total IgA was measured to exclude IgA deficiency. Thereafter, IgA antiendomysial antibodies were measured using indirect fluorescent antibody test anti-EmA (monkey endomysial) IgA Assay (Scimedx Corporation, Denville, New Jersey, USA) according to the instructions of the manufacturer. A titre of 1:10 U/l or more was considered positive.

A total of 163 patients (108 women, 55 men, median age 35 years, range 16–75 years) fulfilled the inclusion criteria and have been included in this analysis. 154 (94%) patients were diagnosed finally with irritable bowel disease, 4 with Crohn's disease, 2 with hyperthyroidism, 1 with endometriosis, 1 with lactase deficiency and 1 with idiopathic bile salt malabsorption.

Fifteen patients were not screened with antiendomysial antibodies. In four of these patients, the treating physician ordered duodenal biopsy specimens because of predominant diarrhoeal symptoms. Histological examination of the biopsy specimens showed normal mucosa in all of them.

Therefore, in 148 evaluated patients antiendomysial antibodies were measured. IgA

deficiency was not observed. Moreover, in none of the 148 patients (0%, 95% confidence interval 0 to 2, 4%) endomysial antibodies were positive. In 32 patients, the treating physician ordered duodenal biopsy specimens as well. In two of them, histological examination of the biopsy specimens showed an increased number of intraepithelial lymphocytes but a normal villous morphology, in the other 30 patients, duodenal biopsy specimens showed normal mucosa.

To our knowledge, only two similar studies have been performed in the Western world.[4 7] Our study is in contrast with a study performed in the UK that observed 11 cases of positive antiendomysial antibodies in 300 patients (3.6%).[4] Furthermore, a study performed in the US found a prevalence of 4% (2/50 patients) when screening of patients with symptoms suggestive of irritable bowel syndrome was performed with antitissue transglutaminase antibodies, although none of these patients had positive antiendomysial antibodies.[7] Most probably, our study is negative because of a low prevalence of coeliac disease in our examined population. In a recent Dutch survey, the overall prevalence of unrecognised coeliac disease in The Netherlands is 1:286.[8] This is lower than the prevalence of 1:100 observed in the UK and of 1:133 observed in the US.[9 10]

In summary, this study shows that in patients referred to a secondary care hospital in The Netherlands because of suggestive irritable bowel syndrome, the prevalence of coeliac disease is low and that screening for coeliac disease in this population is ineffective.

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## References

1 **Hungin AP**, Whorwell PJ, Tack, J, et al. The prevalence, patterns and impact of irritable bowel syndrome: an international survey of 40 000 subjects. *Aliment Pharmacol Ther* 2003;**17**:643–50.
2 **Drossman DA**, Camilleri M, Mayer EA, *et al.* AGA technical review on irritable bowel syndrome. *Gastroenterology* 2002;**123**:2108–31.
3 **O'Leary C**, Wieneke P, Buckley S, *et al.* Celiac disease and irritable bowel-type symptoms. *Am J Gastroenterol* 2002;**97**:1463–7.
4 **Sanders DS**, Carter MJ, Hurlstone DP, *et al.* Association of adult celiac disease with irritable bowel syndrome: a case–control study in patients fulfilling ROME II criteria referred to secondary care. *Lancet* 2001;**358**:1504–8.
5 **Spiegel BM**, DeRosa VP, Gralnek IM, *et al.* Testing for celiac sprue in irritable bowel syndrome with predominant diarrhea: a cost-effectiveness analysis. *Gastroenterology* 2004;**126**:1721–32.
6 **Mein SM**, Ladabaum U. Serological testing for coeliac disease in patients with symptoms of irritable bowel syndrome: a cost-effectiveness analysis. *Aliment Pharmacol Ther* 2004;**19**:1199–210.
7 **Locke GR 3rd**, Murray JA, Zinsmeister AR, *et al.* Celiac disease serology in irritable bowel syndrome and dyspepsia: a population-based case–control study. *Mayo Clin Proc* 2004;**79**:476–82.
8 **Schweizer JJ**, von Blomberg BME, Bueno-de Mesquita HB, *et al.* Coeliac disease in the Netherlands. *Scand J Gastroenterol* 2004;**39**:359–64.
9 **McLoughlin R**, Sebastian SS, Qasim A, *et al.* Coeliac disease in Europe. *Aliment Pharmacol Ther* 2003;**18**:45–8.
10 **Accomando S**, Cataldo F. The global village of celiac disease. *Dig Dis Sci* 2004;**36**:492–8.

## Functional polymorphisms in the promoters of *MMP-1*, *MMP-2*, *MMP-3*, *MMP-9*, *MMP-12* and *MMP-13* are not associated with hepatocellular carcinoma risk

The matrix metalloproteinases (MMPs) play an important role in several steps of cancer development by regulating cancer-cell growth, differentiation, apoptosis, invasion, metastasis, angiogenesis and immune surveillance.[1] Several polymorphisms in the promoters of a number of *MMP* genes, which are thought to affect the respective MMP production in an allele-specific manner, have been well characterised.[2–4] There is increasing evidence indicating that these functional polymorphisms may contribute to interindividual differences in susceptibility to a wide spectrum of cancers.[2–7] The role of the *MMPs* polymorphisms in hepatocellular carcinoma (HCC), however, has never been specifically investigated. As *MMPs* are plausible HCC candidate genes, we sought to examine whether the *MMP* polymorphisms have any bearing on the risk of HCC. Among the candidate polymorphisms, we focused on seven in the promoters of six *MMP* genes. These polymorphisms were *MMP-1* -1607 1G/2G (rs1799750), *MMP-2* C-1306T (rs243865) and C-735T (rs2285053), *MMP-3* -1612 5A/6A (rs3025058), *MMP-9* C-1562T (rs3918242), *MMP-12* G-82A (rs2276109), and *MMP-13* G-77A (rs17860523), respectively.[2–7]

We genotyped these seven polymorphisms in 434 incident patients with HCC and 480 controls enrolled at Fusui County and its surrounding regions at Guangxi province, a well-known high-risk region for HCC located in southern China. The diagnosis of cases, the inclusion and exclusion criteria for cases and controls and the definition of hepatitis B virus (HBV) carriers, smokers and drinkers were described previously.[8] All seven polymorphisms were genotyped by DNA sequencing. The primers and conditions used for amplifying and sequencing the target region containing these seven polymorphisms are available on request. Haplotypes were assigned by the PHASE (http://www.stat.washington.edu/stephens/). The fitness to Hardy–Weinberg equilibrium was tested using the $\chi^2$ test. The association between the genotypes/haplotypes and HCC risk was evaluated by multiple logistic regression analyses while controlling for confounding factors (including age, sex, status of smoking and drinking, pack-years of smoking and family history of HCC). Potential modification effect of the polymorphisms on HCC risk was assessed for the above risk factors by the addition of interaction terms in the logistic model and by separate analyses of subgroups of subjects determined by these factors.

The controls were comparable with cases with regard to age, status of smoking and drinking, and pack-years of smoking ($p>0.05$). However, more men ($p = 0.005$), HBV carriers ($p<0.001$) and patients with a history of HCC in their first-degree biological relatives ($p<0.001$) presented in cases than in controls. The observed genotype frequencies for the seven polymorphisms conformed to the Hardy–Weinberg equilibrium in both cases and controls ($p>0.05$). On the basis of logistic regression analysis with adjustment for confounding factors, no significant association with the risk of HCC was observed with the seven polymorphisms in the overall sample, and HBV carriers and non-HBV carriers (table 1). The distribution of haplotypes based on the *MMP-2* C-1306T and C-735T polymorphism exhibited no significant difference between the cases and controls (data not shown). When the analyses were stratified by age, sex, status of smoking and drinking, pack-years of smoking and family history of HCC, no significant association was found between the *MMP* polymorphisms/haplotypes and the risk of HCC (data not shown).

There are several possible reasons for our negative results. First, inadequate power may be an explanation of our results. However, this study had >85% power at a significance of 0.05 to detect a recessive allele with a minor allele frequency of 0.20 that confers a risk of 1.4. Second, our findings could be the result of the different molecular mechanisms of HCC and other types of cancer. Alternatively, there may be population-specific differences in the contribution of these *MMPs* polymorphisms to HCC susceptibility. This might occur if there were population differences in linkage disequilibrium patterns or allele frequencies of *MMP* genes. Indeed, the allele and genotype frequencies of *MMP* polymorphisms vary with ethnicity. For instance, in this study with 480 control subjects, we found that the *MMP-2* -1306C allele frequency was 0.92 and that the C/C genotype accounted for 85.0% of the southern Han Chinese population studied, compared with around 0.75 and 60.0%, respectively, among Caucasians from France and the UK.[4 9] Finally, our negative results may be due to inherent selection bias. As a hospital-based study, our patients with HCC were enrolled from the hospitals and the controls were selected from the community population, and therefore inherent selection bias cannot be completely excluded. However, by matching for age and residential area, potential confounding factors might have been minimised and any inadequacy in matching have been controlled in data analyses with further adjustment and stratification.

In conclusion, to the best of our knowledge, this is the first case–control study of *MMP* polymorphisms in relation to HCC. Our results suggest that the seven functional polymorphisms in the promoters of *MMP-1*, *MMP-2*, *MMP-3*, *MMP-9*, *MMP-12* and *MMP-13* do not significantly confer an increased risk of HCC. However, additional studies from larger populations among Chinese, and from diverse ethnic populations, are warranted before the importance of *MMP* polymorphisms in HCC risk can be fully ascertained.

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