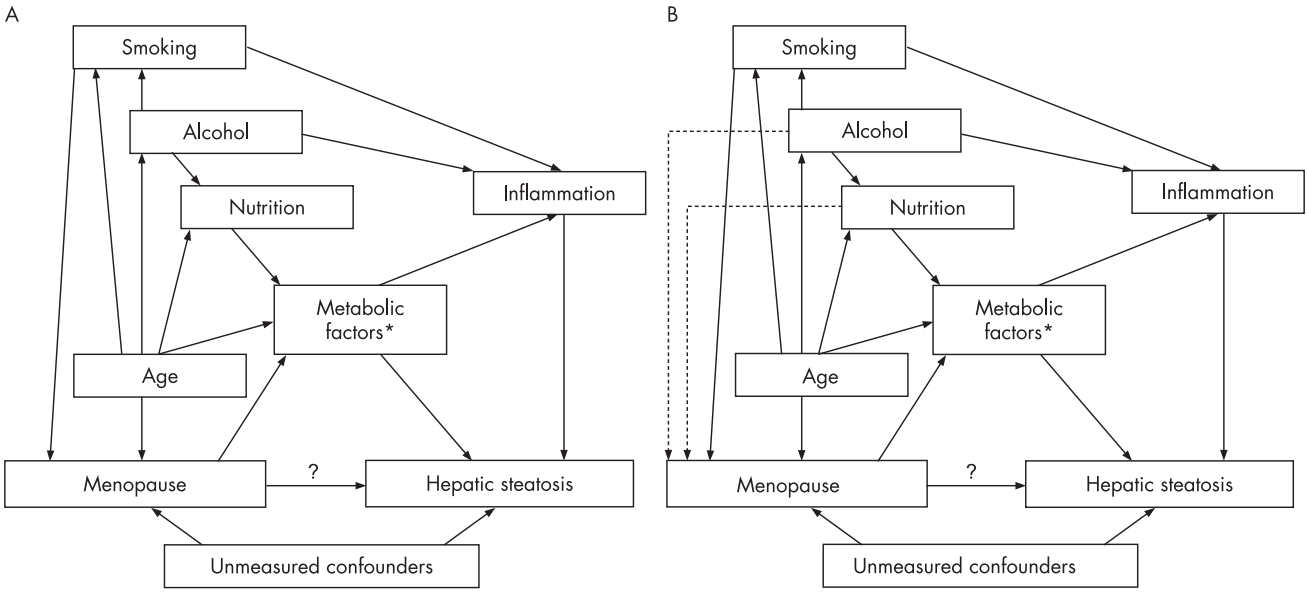

Figure 1 Causal diagram for the association between menopause and hepatic steatosis. (A) A minimum set of confounders would include age, smoking status and unmeasured confounders such as common genetic factors. (B) In contrast with (A), the diagram considers additional causal effects of alcohol consumption and nutrition on menopausal status (dotted arrows). In this scenario, a minimum set of confounders would include age, smoking status, nutritional factors, alcohol consumption and unmeasured confounders such as common genetic factors.

status and hepatic steatosis found in our study may thus reflect the changes in fat distribution during menopausal transition.

Causal diagrams enable the accurate selection of covariables from a variety of potential variables influencing the studied association. It has been shown that an arbitrary inclusion of variables in multivariable models may not only lead to an underestimation of the association of interest but also introduce bias in the analyses.[5] According to our directed acyclic graphs, metabolic factors should be regarded as mediators rather than confounders for the relationship investigated.

We conclude that menopausal status is associated with hepatic steatosis. The prognostic relevance of this association with respect to incident cardiovascular disease and diabetes has to be proved.

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Association between *Helicobacter pylori* infection and interleukin 1β polymorphism predispose to CpG island methylation in gastric cancer

Interleukin1β (IL1β) is upregulated in the presence of *Helicobacter pylori* infection.[1] IL1β polymorphisms with T/T and T/C genotypes enhance IL1β production, and are associated with an increased risk of *H pylori*-induced hypochlorhydria[2] and gastric cancer.[3] The relationship between *H pylori* and CpG island methylation has been repeatedly reported.[4–6] It has been reported that IL1β can modulate CpG island methylation through activation of DNA methyltransferase and hence repress gene expression.[7] We therefore hypothesised that patients with *H pylori* infection and IL1 polymorphism, by the production of IL1β, are predisposed to gastric cancer development through the CpG island methylation pathway.

We obtained surgical specimens and their corresponding peripheral blood from 98 consecutive patients with gastric cancer admitted to Queen Mary Hospital, Hong Kong. This study was approved by the ethics committee. The methylation status of the death-associated protein-kinase, O^6-methyl-guanine methyl-

Table 1 Correlation between methylation at two or more genes and IL1β-511 genotype, and other tumour characteristics

	Less than two genes methylated	Two or more genes methylated	OR	95% CI
Locus at IL1β-511 (whole group, n=98), overall p=0.02				
C/C	56.2% (9)	43.8% (7)	1	
C/T	25.9% (15)	74.1% (43)	3.7	1.2 to 12.1
T/T	16.7% (4)	83.3% (20)	6.4	1.6 to 30.7
Locus at IL1β-511 (*H pylori* positive, n=64) overall p=0.03				
C/C	62.5% (5)	37.5% (3)	1	
C/T	23.1% (9)	76.9% (30)	5.6	1.2 to 31.7
T/T	11.8% (2)	88.2% (15)	12.5	1.8 to 125.7
Locus at IL1β-511 (*H pylori* negative, n=34), overall p=0.61				
C/C	50% (4)	50% (4)	1	
C/T	31.6% (6)	68.4% (13)	2.2	0.4 to 12.4
T/T	28.6% (2)	71.4% (5)	2.5	0.3 to 25.9
H pylori status, overall p=0.29				
+	25% (16)	75% (48)	1.6	0.7 to 4
−	35% (12)	65% (22)	1	
Patient age (years), overall p=0.44				
⩽60	23% (7)	77% (23)	1	
>60	31% (21)	69% (47)	0.7	0.2 to 1.7
Tumour stage, overall p=0.004				
I and II	12.8% (5)	87.2% (34)		
III and IV	39% (23)	61% (36)	0.23	0.07 to 0.63
Anatomical site, overall p=0.55				
Antrum	27% (20)	73% (54)	1	
Non-antrum	33.3% (8)	66.7% (16)	0.7	0.3 to 2
Histology (Lauren's classification), overall p=0.09				
Intestinal	21.2% (11)	78.8% (42)	1	
Diffuse	32.3% (10)	67.7% (21)	0.55	0.2 to 1.5
Mixed	50.0% (7)	50.0% (7)	0.26	0.1 to 0.9
Methylation status stratified by genotype				
	H pylori positive	*H pylori* negative		
C/C	62.5% (5/8)	50% (4/8)	0.6	0.07 to 4.4 (p=0.61)
C/T	23% (9/39)	68% (13/19)	1.5	0.4 to 5.2 (p=0.49)
T/T	11.7% (2/17)	71% (5/7)	3	0.3 to 31.1 (p=0.33)

*ORs of two or more genes methylated, with the first group being the referent group.

transferase, p16 genes[8] and E-cadherin[9] promoter was determined by methylation-specific polymerase chain reaction. Genotyping of IL1 polymorphism was performed as reported previously.[2 10] *H pylori* status was determined by serology using a commercially available ELISA kit (pylori DTect ELISA, Diagnostic Technology Pty, New South Wales, Australia) and histology using Giemsa stain. *H pylori* status was considered to be positive if either test showed a positive result.

*H pylori i*nfection was present in 65% (64) of patients. In the IL1β gene, the T and C alleles at the -511 locus of the IL1β gene were in near total linkage disequilibrium with the C and T alleles at the -31 locus. Analysis was therefore restricted to the IL1β-511 locus, but the associations with the -31 locus were identical. The allele frequencies at IL1β-511 were 16% (16), 59% (58) and 25% (24) for C/C, C/T and T/T genotypes, respectively. Methylation of methylguanine-DNA methyltransferase, E-cadherin, death-associated protein and p16 was present in 30% (29), 59% (58), 43% (42) and 45% (44) of patients, respectively (examples shown in fig 1). Patients with the T/T or T/C genotype showed an increased odds ratio (OR) of 6.4 and 3.7, respectively, with respect to the C/C genotype for developing methylation at two or more genes (table 1). This OR for developing methylation at two or more genes was markedly increased in patients with *H pylori* infection and with the T/T (12.5) or T/C (5.6) genotype with respect to the C/C genotype but was not present in patients without *H pylori* infection. However, no association of the genotype on an individual methylation marker was seen (table 1). Also, there was no association between the number of genes methylated and *H pylori* status, no difference in age between those with or without methylation at two or more genes, and anatomical site, except that lower stages (stages I and II) were associated with more frequent methylation at two or more genes (table 1).

The underlying mechanisms or the environmental factors governing the simultaneous methylation of multiple genes in gastric cancer are still unclear. Hmadcha *et al*[7] have reported gene silencing due to methylation at the CpG island in the presence of IL1β. We have previously reported that eradication of *H pylori* may result in reversal of methylation at tumour suppressor genes in non-lesional gastric mucosa.[5] Our study may further support the idea that *H pylori* infection stimulates the production of IL1β, and the presence of the proinflammatory T allele further enhances the production of IL1β. The synergistic effect of these two factors was observed in our study to correlate with the increased frequency of methylated genes. Thus, patients with *H pylori* infection and IL1β-511 T/T genotype may be predisposed to gastric cancer through the CpG island methylation pathway.

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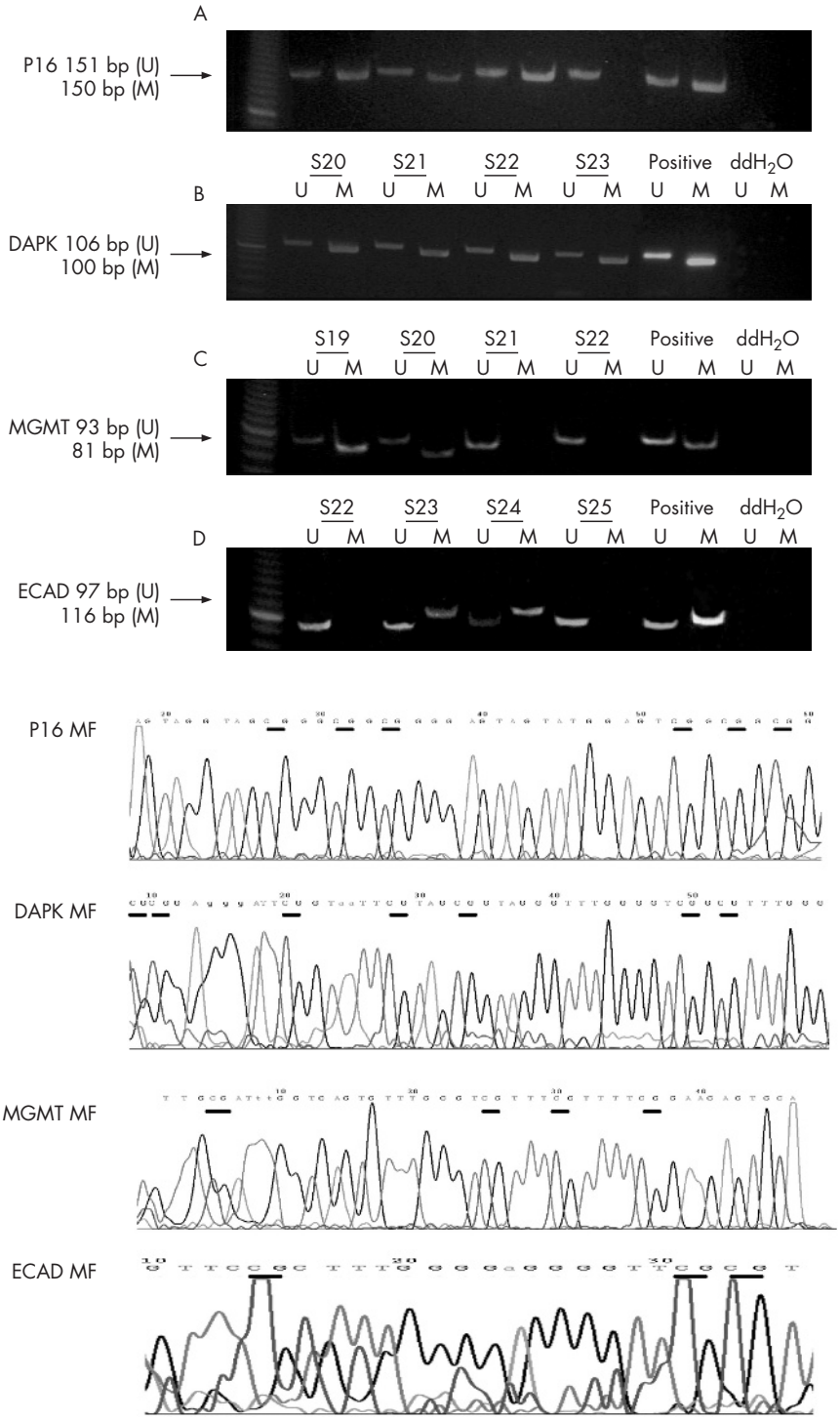

Figure 1 Examples of polymerase chain reaction products of P16, DAPK, E-cadherin (ECAD) and O^2-methyl-guanine methyl transferase (MGMT) genes. The lower panel shows segments of the methylated sequences of these gene products. Methylated cytosine residues that are unchanged are shown as underlined.

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Restricted use of albumin for spontaneous bacterial peritonitis

Spontaneous bacterial peritonitis (SBP) may precipitate deterioration of circulatory function with severe hepatic insufficiency, hepatic encephalopathy, and type-1 hepatorenal syndrome (HRS) and has 30% hospital mortality despite infection resolution.[1] Predictors of this acute-on-chronic liver failure include ascitic fluid concentrations of granulocytes and cytokines and renal and hepatic insufficiency at diagnosis.[1–3] Endotoxemia and the inflammatory response precipitate renal failure (RF) by accentuating splanchnic vasodilatation and impairing cardiac function.[3–5] Compensatory activation of the renin-angiotensin and sympathetic nervous systems further decrease renal perfusion. Volume expansion with albumin (1.5 g/kg day one, 1 g/kg day three) significantly reduces the incidence of HRS and hospital mortality.[2]

In the sole reported trial, only patients with serum bilirubin (bili) >68.4 μmol/l, blood urea nitrogen (BUN) >30 mg/dl or serum creatinine (Cr) >88.4 μmol/l appeared to benefit from albumin.[2] In this report we describe a therapeutic protocol involving 38 episodes in 28 patients at the Mount Sinai Medical Center New York and the Barcelona Hospital Clinic in which albumin (1.5 g/Kg on the day of diagnosis, 1 g/Kg on the third day; Human Albumin 25%, ZBL Bioplasma AG, Berne, Switzerland) was restricted to those at high risk for RF (bili > 68.4 μmol/l or Cr >88.4 μmol/l) (table 1). Diagnosis and treatment of SBP were based on established guidelines.[6] Cardiovascular and splanchnic hemodynamics and the activity of the renin-angiotensin system and inflammatory response were assessed in 6 low-risk cases from Barcelona at SBP diagnosis and resolution.[3 7 8]