PostScript

LETTERS

Analysis of inherited MYH/ (MutYH) mutations in British Asian patients with colorectal cancer

Biallelic inherited mutations of the MYH gene (also known as MutYH or human MutY homologue) are associated with multiple colorectal adenomas and a high risk of colorectal cancer that approaches 100%.^{1,2} This recessive disorder has become known as MYH-associated polyposis (MAP) to distinguish it from dominantly inherited familial adenomatous polyposis. The risk of colorectal cancer in heterozygotes seems to be only marginally increased, if at all.² During a review of the Wales Polyposis Register we noted that although only 4 of the 115 recorded families were of Asian origin, all four had MAP. This was in contrast to the 111 indigenous families, only 12 of which had MAP. All affected members of three unrelated British Indian families were homozygous for the mutation E466X and one patient of Pakistani descent was homozygous for Y90X.³ These mutations have not been observed in other ethnic groups. As the overall incidence of colorectal cancer among Asians living in England and Wales is markedly below that of the general population,⁴ we postulated that MYH mutations might contribute more significantly to colorectal cancer among British Asians than among indigenous northern Europeans. We conducted a retrospective study to assess the contribution of MYH mutations to colorectal cancer among British Asians.

Multicentre research ethics Committee approval was obtained (Wales MREC 03/9/033). Cases were ascertained through departments of pathology at five participating UK centres, three in Wales and two centres in Leicester and Birmingham, which were selected for their large Asian populations. In each centre, we identified archived paraffin-wax-embedded blocks of tissue from cases of colorectal cancer by searching by forename and surname for cases of Indian subcontinent origin.^{5,6} All cases were anonymised before despatch for laboratory analysis. Of the 143 identified cases, 120 yielded DNA suitable for polymerase chain reaction (PCR) amplification. From the same centres, 174 control paraffin-wax-embedded colonic mucosa samples were also identified from individuals with names of Indian subcontinent origin, in whom colonic biopsy specimens had revealed a histopathological diagnosis of inflammation without neoplasia. Of these, 100 yielded DNA suitable for PCR analysis. In view of the difficulties inherent in obtaining adequate numbers of samples with a relatively rare condition in a minority ethnic group, we did not attempt to age match the groups. All samples in the case group were subjected to histopathological re-examination to ensure analysis of only normal background tissue. PCR and restriction enzyme digestion were used to identify of the Asian mutations E466X (using *ApoI*) and Y90X (using *RsaI*), and also to identify the common northern European mutation G382D (using *BglII*).

Amplification refractory mutation system PCR was used to detect the second most common European mutation, Y165C (details of primers and PCR conditions are available online at (http://www.cardiff.ac.uk/medicine/medical_genetics/genetics_research/tmg/projects/Dolwani2006/). Positive results were confirmed by bidirectional sequencing (ABI Prism 3100). Raw data for all phenotypic features wherever available and results in all cases and controls is given in supplementary table A (available at <http://www.gutjnl.com/supplemental>).

One case (1/120) and one control (1/100) sample were found to be heterozygous for the E466X variant of MYH. No cases or controls had biallelic mutations or carried any of the other mutations tested (Y165C, G382D and Y90X). Sequencing of the rest of the coding region was possible only for exons 2, 3, 11, 14 and 16 of MYH in these two individuals, and did not reveal any other mutation. Owing to the nature of the ethical approval, we were unable to follow up the two heterozygotes for further sampling and more details of phenotype or family history apart from that mentioned. We conclude that these four MYH mutations are unlikely to be a major contributor to colorectal cancer in British Asians. A much larger series would be required to quantify accurately the proportion of colorectal cancer attributable to MYH in this group. Nonetheless, previous studies have identified homozygotes for the E466X allele of MYH in British Asians with MAP, and our observation of this allele in the heterozygous state both in unselected cases and controls strengthens the case for initial testing for this mutation in patients from this population with polyposis or apparently recessive colorectal cancer.^{2,3,7} Studies in other ethnic subgroups have also suggested that the identification and characterisation of population-specific ancestral variants such as 1395delGGA in Italians^{8,9} will be helpful for the provision of molecular diagnostics for MAP.

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Proton pump inhibitor-therapy refractory gastro-oesophageal reflux disease patients, who are they?

In a recent version of *Gut*, Mainie and colleagues report on patients with persistent symptoms despite acid suppressive therapy.¹ This is often presented as a considerable problem and impedance seems useful in the evaluation of these patients. The importance of the phenomenon of proton pump inhibitor (PPI)-refractory symptoms is also stressed in studies in which the need for a new anti-reflux drug is propagated. However, we think some caution is appropriate when speaking of PPI-refractory gastro-oesophageal reflux disease (GORD) symptoms, in particular when it is decided that new reflux-inhibiting drugs are warranted to treat the “PPI-resistant reflux symptoms”.

Firstly, the term PPI-resistant GORD is often used for patients that are not fully investigated,

particularly with regard to the question whether their symptoms truly originate from reflux of gastric contents. Indeed, what are judged "reflux symptoms" does not necessarily imply that these are due to GORD, as they may also occur in conditions such as functional heartburn and functional dyspepsia. Reduction of reflux episodes with a new drug will not result in symptom reduction in these latter patients.

Secondly, when one has ascertained that a patient's symptoms are indeed caused by gastro-oesophageal reflux, it is pivotal to treat the patient for a sufficient period of time. The effect of a PPI on acid secretion is within days but due to acid-induced oesophageal hypersensitivity the number of responders seems to increase for up to 8 or even 12 weeks of acid suppressive treatment.^{2,3} Compliance to drug intake may be an issue when symptoms do not resolve immediately after onset of therapy. When symptoms persist even after a sufficiently long treatment period, either the drug does not adequately inhibit acid secretion and a higher dose is required, or the symptoms are induced by weak levels of acid (above pH 4), or the non-acid component of the reflux in which the only option would be a reduction of reflux episodes (by pharmacotherapeutic or surgical means).⁴ These patients are the ones that can be identified by impedance monitoring as Mainie *et al.* show in their study. These are also the patients that would benefit from a future reflux-reducing drug.

We conclude that a critical approach is required when symptoms do not respond adequately to PPI therapy in suspected GORD patients. The true number of PPI-refractory GORD patients is likely to be overestimated.

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Menopausal status and hepatic steatosis in a general female population

Population-based studies showed that non-alcoholic hepatic steatosis is less common in women than in men. Anti-oestrogens double the risk of non-alcoholic steatohepatitis,¹ which may be interpreted as indirect evidence for a protective role of endogenous oestrogens against steatohepatitis. Experimental evidence further suggests that oestradiol causes a reduction of hepatic steatosis in mice unable to produce oestrogens.² We thus hypothesised that the menopause as a natural state of oestrogen deficiency might increase the risk of hepatic steatosis.

The Study of Health in Pomerania is a cross-sectional population-based survey in northeast Germany.³ We restricted our analyses to 808 women aged 40-59 years without seropositivity to hepatitis B antigen and anti-hepatitis C virus. The study was approved by the ethics committee of the University of Greifswald. All participants gave written informed consent.

Menopause was defined as 12 consecutive months of amenorrhoea or cessation of menstruation after iatrogenic intervention. Serum

glutamate oxaloacetate transaminase, glutamic pyruvic transaminase and γ glutamyl transpeptidase levels were measured photometrically. Levels exceeding the 75th centile for each marker were considered to be raised. In liver ultrasound, a hypoechoic pattern was defined as the presence of a bright pattern with contrast between hepatic and renal parenchyma.⁴ Hepatic steatosis was defined as the presence of a hypoechoic ultrasound pattern and at least two raised levels among three serum transaminase levels. Multivariable statistical analyses were performed by logistic regressions. Sets of confounders were defined using directed acyclic graphs.⁵

Descriptive statistics showed the association between menopausal status and liver-related characteristics, which remained stable after adjustment for age (table 1). A first sufficient set of covariables included age, smoking and unmeasured confounders (fig 1A). Controlling analyses for age and smoking only marginally affected the relationship investigated (table 1, model 1). In a second directed acyclic graph (fig 1B), alcohol use and nutritional factors were further assumed as determinants of hepatic steatosis and also included in the sufficient set of covariables. This extended model did not substantially affect the association of interest (table 1, model 2). Model 2 was then supplemented by covariables without using any causal diagrams. These analyses gave non-conclusive results (table 1, model 3). Exclusion of all women with alcohol consumption >20 g/day ($n = 122$; 15.1%) changed the major results only marginally.

Our findings are in good agreement with a Korean ultrasound study⁶ in which the menopause was also a determinant of hepatic steatosis in women. Our results are further consistent with analyses of the National Health and Nutrition Examination Survey III.⁷ Non-alcoholic hepatic steatosis as defined by increased concentrations of liver enzymes was twice as common in postmenopausal as in premenopausal women.⁷

Oestrogens lead to a preferential fat accumulation in the gluteofemoral region⁸ and, conversely, the loss of oestrogens during menopause transition is associated with an increase in central fat.⁹ In obese women with hepatic steatosis, liver volumes correlate highly with visceral adipose tissue.¹⁰ The relationship between menopausal

Table 1 Relationship between menopausal status and liver-related characteristics

	Hypoechoic liver pattern	Raised serum GOT	Raised serum GPT	Raised serum GGT	Hepatic steatosis
Menopause, crude	2.41 (1.71 to 3.38)*	2.38 (1.68 to 3.36)*	2.53 (1.80 to 3.57)*	1.61 (1.16 to 2.24)*	5.88 (4.34 to 7.95)*
Menopause, adjusted for age	1.70 (1.1 to 2.61)*	1.75 (1.12 to 2.72)*	2.13 (1.37 to 3.31)*	1.62 (1.06 to 2.48)*	2.51 (1.57 to 4.02)*
Menopause, adjusted for model 1	1.71 (1.11 to 2.63)*	1.79 (1.14 to 2.79)*	2.15 (1.38 to 3.36)*	1.55 (1.01 to 2.38)*	2.51 (1.56 to 4.02)*
Menopause, adjusted for model 2	1.71 (1.11 to 2.64)*	1.78 (1.14 to 2.78)*	2.14 (1.37 to 3.33)*	1.56 (1.01 to 2.41)*	2.47 (1.54 to 4)*
Menopause, adjusted for model 3	1.45 (0.87 to 2.39)	1.67 (1.04 to 2.68)*	2.01 (1.23 to 3.26)*	1.43 (0.90 to 2.28)	1.93 (1.13 to 3.31)*

GGT, γ glutamyltransferase; GOT, glutamic-oxaloacetic transaminase; GPT, glutamic-pyruvic transferase.

Logistic regression analysis. Data are OR (95% CI).

Model 1 included age and smoking status (three categories) as sufficient set of confounders as defined from the causal diagram in fig 1.

Model 2 included age, smoking status (three categories), nutrition (three categories) and alcohol consumption (three categories) as defined from an alternate causal diagram in fig 2.

Model 3 included variables of model 2 and, in addition, body mass index (three categories), diabetes, use of menopausal hormone treatment, increased waist-to-hip ratio as well as increased serum low-density lipid cholesterol and plasma fibrinogen levels.

Hepatic steatosis was defined as the presence of a hypoechoic liver pattern in ultrasound and at least two among three characteristic raised serum GOT, GPT or GGT levels.

* $p < 0.05$.