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

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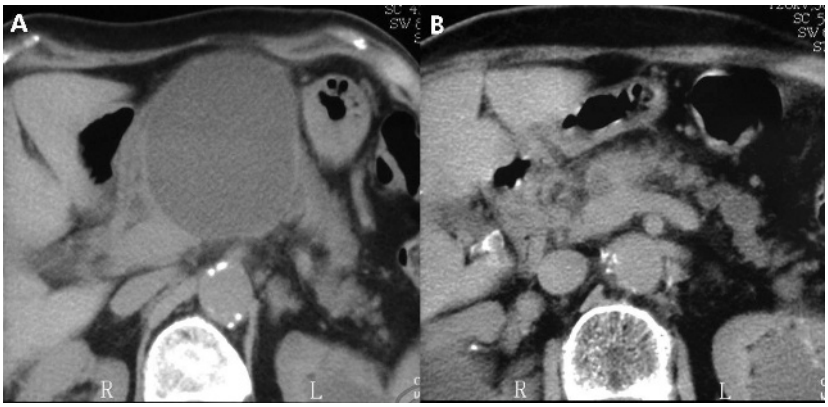

Figure 1 (A) Computed tomography scan showing an 8 cm pancreatic body cyst. (B) Cyst regression at six months after lavage with povidone iodine.

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 iodate 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 iodate. 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 ethanol[8] 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 ($p<1\times10^{-5}$). 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 ($p<1\times10^{-5}$). 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) chemotherapy[7–10] 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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[−215G>A; IVS3+2T>C] mutation in the *SPINK1* gene causes exon 3 skipping and loss of the trypsin binding site

Previous studies have shown an association between chronic pancreatitis (CP) and mutations, especially the N34S mutation, in the serine protease inhibitor Kazal type 1 (*SPINK1*) gene.[1 2] The human *SPINK1* gene is approximately 7.5 kb long and consists of four exons.[3] The gene product consists of 79 amino acids, including a 23 amino acid signal peptide. In exon 3, SPINK1 possesses a reactive site that serves as a specific target substrate for trypsin.[4] It has been suggested that *SPINK1* mutations might result in altered interaction between SPINK1 and trypsin, thus affecting the protease/antiprotease balance within the pancreas.[1 2] But the underlying molecular mechanisms remain unclear. Splicing defects are estimated to account for approximately 10–15% of disease causing mutations in humans.[5] Changes in the splicing patterns and in levels of normal transcripts lead to phenotypic differences. The prevalence of splicing mutations in the *SPINK1* gene is unknown. Most reported mutations have only been described at the DNA level and have not been studied at the mRNA level, mainly due to unavailability of *SPINK1* mRNA from patients.

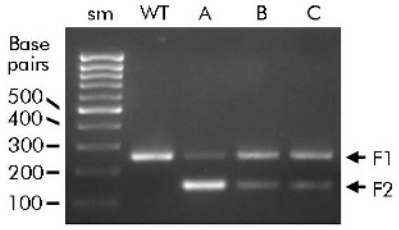

Figure 1 [−215G>A; IVS3+2] mutation produced a truncated transcript. Total RNA was isolated from the biopsy specimen of the stomach, and the entire coding region of the serine protease inhibitor Kazal type 1 (*SPINK1*) gene was amplified by reverse transcription polymerase chain reaction, followed by 2 % agarose gel electrophoresis. Sm, size marker (100 base pair ladders), WT, healthy control. Patient A with alcoholic chronic pancreatitis (CP) was homozygous for the [−215G>A; IVS3+2T>C] mutation. His daughter B and patient C with idiopathic CP were heterozygous. In subjects carrying the [−215G>A; IVS3+2T>C] mutation, two bands were observed: a fragment corresponding to a normal (''F1'') and a truncated (''F2'') band.

We have recently shown that the [−215G>A; IVS3+2T>C] mutation is associated with familial and idiopathic CP in Japan.[6 7] Because the IVS3+2T>C mutation affects the consensus splicing donor site,[8] we hypothesised that this mutation leads to alternative splicing, resulting in decreased SPINK1 function. To overcome the difficulties in obtaining human pancreas samples, *SPINK1* mRNA was harvested from the stomach, where SPINK1 is also abundantly expressed,[9] of (1) a 70 year old male A with alcoholic CP carrying the homozygous [−215G>A; IVS3+2T>C] mutation, (2) his 40 year old daughter B, a heterozygote who had no abdominal complaints to date, and (3) a 54 year old female C with idiopathic CP, also heterozygous for the [−215G>A; IVS3+2T>C] mutation. Total RNA was isolated from biopsy specimen of the stomach. The entire coding region of the *SPINK1* gene was amplified by reverse transcription-polymerase chain reaction (PCR) and sequenced. Electrophoresis of the reverse transcription PCR products from subjects carrying the [−215G>A; IVS3+2T>C] mutation revealed two bands: a fragment corresponding to a normal (''F1'') and a truncated (''F2'') band (fig 1). Sequencing of the truncated fragment revealed complete deletion of exon 3. This mutated protein was predicted to consist of 63 amino acids: deletion of amino acid sequence from residues 30–64 and shifting of the reading frame at amino acid 65.

To our knowledge, this is the first study showing the splicing problem in the *SPINK1* gene at the mRNA level. Northern blot analysis revealed that the size of the *SPINK1* transcript was identical both in the pancreas and stomach,[9] suggesting that exon 3 skipping is also likely to occur in the pancreas. It is logical to assume that skipping of exon 3 would result in functional loss of SPINK1, thus affecting the protease/antiprotease balance within the pancreas. Of note, the daughter of patient A carrying the heterozygous [−215G>A; IVS3+2T>C] mutation has not yet developed CP. Because this mutation has not been found in healthy controls,[7] it is of interest to see whether she will develop CP in the future. Recently, Le Marechal and colleagues[10] reported the IVS2+1G>A mutation in a CP patient carrying the P55S mutation in France. The IVS2+1G>A mutation affects the consensus splicing donor site of intron 2, implying a role of another splicing variation. Further studies using larger numbers of patients and different types of mutations will establish the role of splicing mutations in SPINK1 related CP.

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Neoadjuvant chemoradiation treatment impairs accuracy of MRI staging in rectal carcinoma

Neoadjuvant chemoradiotherapy (nCRT) is considered one of the treatment modalities of advanced rectal cancer (pT3/T4 or pN+) with the intention of downsizing and downstaging the tumour. Tumour restaging may be useful for planning the operation but tissue alteration after nCRT may disturb the accuracy of the imaging procedures.

Between July 2004 and August 2005, we analysed 28 consecutive patients (18 males, 10 females, ~63 years) with adenocarcinoma of the middle and distal third of the rectum. High spatial resolution magnet resonance imaging (MRI) with intraluminary contrast and endorectal ultrasonography (EUS) (Olympus EU-M30S, 12 MHz) were performed before and after nCRT as part of their