LETTER TO JMG

MDM2 SNP309 accelerates colorectal tumour formation in women

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Recent studies have shown that the G-allele of MDM2 SNP309 (T/G) in the *p53* tumour suppressor pathway can accelerate tumorigenesis and alter the risk of various cancers in women and not in men. In this report, data are presented from two independent groups of patients that suggest that the G-allele of SNP309 accelerates colorectal tumour formation only in women, and that lend further support to the model that primarily female-specific hormones, such as oestrogen, could either directly or indirectly allow for the G-allele of SNP309 to accelerate tumour formation in women.

Key points

- Recent studies have shown that the G-allele of MDM2 SNP309 (T/G) in the *p53* tumour suppressor pathway can accelerate tumorigenesis and alter the risk of various cancers in women and not in men.[1 2] A model was proposed that, primarily, female-specific hormones, such as oestrogen, could either, directly or indirectly, allow the G-allele of SNP309 to accelerate tumour formation in women.[1] In this report, two independent groups of patients were studied to determine whether the G-allele of MDM2 SNP309 could also accelerate colorectal tumour formation in a sex-specific and hormone-dependent manner.
- In 165 patients with colorectal cancer, women, but not men, who carried the G-allele of SNP309 in either the heterozygotic or the homozygotic state, showed a significant 9-year average, and 10-year median, earlier age of tumour diagnosis ($p = 0.0013$). These data support the hypothesis that the G-allele of SNP309 is indeed associated with a significant earlier colorectal tumour diagnosis in women, but not in men, as had been suggested by a previous study.[3]
- In two independent groups of patients, women diagnosed with colorectal cancer when levels of primarily female-specific hormones, such as oestrogen, were greater (ie, women at a younger or pre-menopausal age) were noticeably enriched in the G-allele of SNP309 compared with patients diagnosed when, primarily, oestrogen levels were noticeably lower (ie, women at an older age or post-menopausal age, and men). These data support the model that, primarily, female-specific hormones, such as oestrogen, could either directly or indirectly allow for the G-allele of SNP309 to accelerate colorectal tumour formation in women.

The importance of the *p53* stress response pathway in the suppression of tumorigenesis in humans has been shown in many types of cancers. One of the strongest pieces of evidence is the very high rate at which the *p53* gene is weakened by somatic mutations in tumours.[4] One of the highest rates of *p53* mutation is found in colorectal cancers (CRC), wherein up to 44% of all tumours harbour a *p53* mutation.[5] Therefore, it is likely, and indeed there is evidence to support, that single-nucleotide polymorphisms that alter the activity of the *p53* stress response pathway can result in the development of CRC in humans.[6–8]

A central node in the *p53* pathway is the MDM2 protein, a direct negative regulator of *p53*.[9] A single-nucleotide polymorphism (SNP309 T/G) was found in the promoter of the *MDM2* gene, whereby the G-allele resulted in higher levels of MDM2 RNA and protein, and was associated with the attenuation of the *p53* pathway both in vitro and in vivo.[10] In a survey study of the possible effects of the SNP309 locus in three different cancers, data were presented that suggested a sex difference in the effect of SNP309 in CRC.[3] Specifically, it was found that the G-allele of SNP309 was enriched in women diagnosed with CRC compared with the men diagnosed with CRC. Secondly, women with the G-allele of SNP309 showed an earlier average age of diagnosis compared with T/T women, whereas no such observation was made in the male patients. Although these results were separately significant, after adjusting for multiple hypotheses testing, the significance of these observations was lost.

Recent studies of the MDM2 SNP309 locus in other cancers have shown that the G-allele of SNP309 is significantly associated with an earlier and greater frequency of tumour formation only in women.[1 2] In one report, it was reasoned that, since the SNP309 locus is in a region of the *MDM2* promoter, which is regulated by hormonal signalling pathways, and the G-allele of SNP309 increases the affinity of a well-described co-transcriptional activator of nuclear hormone receptors, such as Sp1, the SNP309 locus could alter the effects of hormones on tumorigenesis.[1] The results of four independent studies of three different sporadic cancers (diffuse large B cell lymphoma, soft tissue sarcoma and invasive ductal breast carcinoma) supported the model that primarily female-specific hormones, such as oestrogen, could either directly or indirectly allow for the G-allele of SNP309 to accelerate tumour formation in women. Similar results have also been seen in a comparison of male and female non-small cell lung cancer and SNP309, whereas only women with the G-allele of SNP309 showed an increased risk for developing lung cancer.[2]

METHODS

Statistical analysis

A one-sided Mann–Whitney U test was used to determine the significance of the age of tumour diagnosis between the groups with and without the G-allele of SNP309. A one-sided

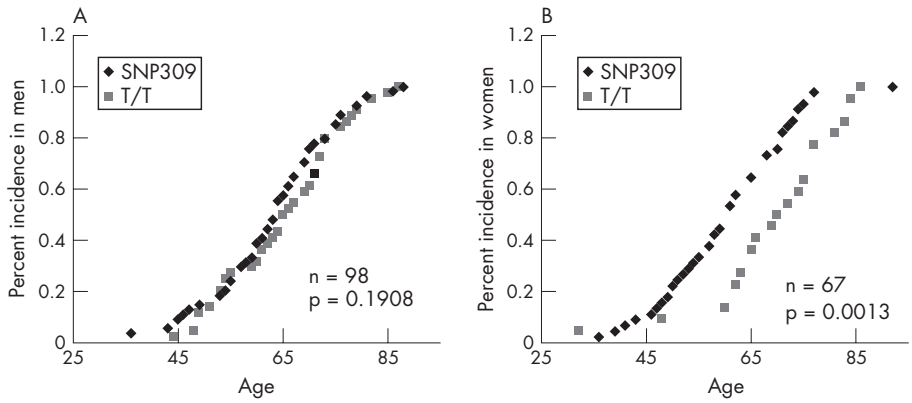

Figure 1 The G-allele of SNP309 associated with an increased age of onset of colorectal cancer with wild-type *p53* genes in women but not in men. The cumulative incidence of colorectal cancer for both the individuals T/T in genotype (black squares) and T/G or G/G in genotype (SNP309, grey diamonds) is plotted as a function of age for men (A) and women (B).

Fisher's exact test was used to determine the significance of the enrichment of patients with the G-allele of SNP309 in the different groups of patients with CRC.

Patients with colorectal cancer (Italy)

Individuals with somatic wild-type *p53* genes were derived from 330 consecutive white Italian patients with colorectal cancer and histologically confirmed colon or rectal adenocarcinoma, who had been treated between 1991 and 1998. In all the 330 patients, exons 4–8 of the *p53* gene were subjected to polymerase chain reaction–single strand conformational polymorphism analysis for point mutations, as previously described.[8]

Patients with colorectal cancer (Finland)

Population-based material from 1042 Finnish patients with colorectal cancer who had been previously screened for MDM2 SNP309 was collected.[3] This well-documented material has been described in more detail in previous reports.

RESULTS AND DISCUSSION

To test whether the G-allele of SNP309 accelerates CRC only in women, a second independent group of 165 Italian patients with CRC, most of whom were genotyped previously,[8] were studied. All these patients had wild-type *p53* genes in their tumours and were diagnosed with CRC on average at the age of 64 years (ranging from 32 to 92 years). Of these 165 patients, 98 were men and 67 were women. When separated into the different genotypes of the SNP309 locus, men showed no significant differences in the average age of tumour diagnosis (T/T men, n = 44, 65 years of age, versus T/G and G/G men, n = 54, 63 years of age, fig 1A, p = 0.191, Mann–Whitney U test). In contrast, women who carried the G-allele, in either the heterozygotic or the homozygotic state, showed a significant 9-year average, and 10-year median, earlier age of CRC onset, compared with women with genotype T/T (n = 22, 70 years of age (ranging from 32 to 86 years) *v* G/G and T/G women, n = 45, 61 years of age (ranging from 36 to 92 years), fig 1B, p = 0.001, Mann–Whitney U test).

This 9-year average, and 10-year median, earlier age of onset of CRC in women with the G-allele of SNP309 compared with T/T women is much larger than the 2.7-year average earlier age of CRC onset that was reported previously.[3] This is probably due to the fact that only patients with CRC who retained wild-type *p53* genes in their tumours were studied here. It had been previously shown that the G-allele of SNP309 associated with an earlier tumour onset only in tumours, which retained wild-type *p53*.[8] One would, therefore, predict that the 2.7-year average earlier age of CRC onset with the G-allele would increase greatly if that study had been restricted to only those patients whose tumours retained wild-type *p53*.

Taken together, these data support the hypothesis that the G-allele of SNP309 does indeed associate with an earlier colorectal tumour diagnosis in women, but not in men, thereby lending further support to the proposed model that primarily, female-specific hormones, such as oestrogen, could

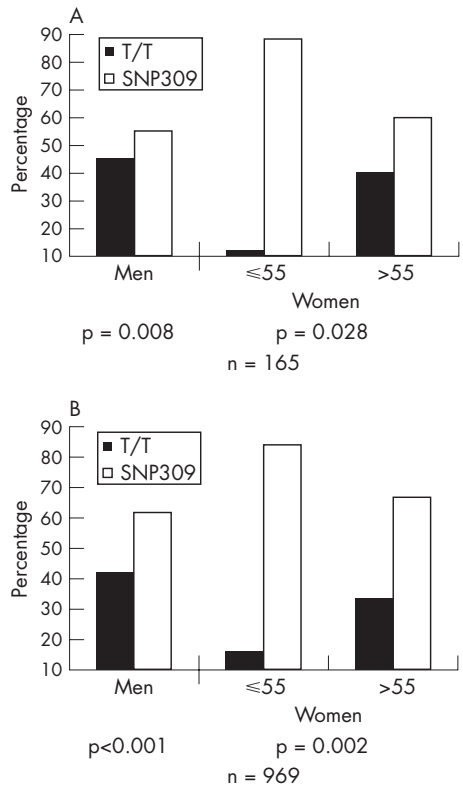

Figure 2 Women with colorectal cancer diagnosed at younger ages are enriched for the G-allele of SNP309. The relative ratios of patients either with the G-allele of SNP309 (SNP309) or without (T/T) are depicted in the bar graphs for the male patients with CRC, the female patients with CRC diagnosed at ⩽*55* years of age and those women diagnosed >*55* years of age for both independent groups of patients with CRC. Panel (A) depicts the group of patients with CRC with wild-type *p53* genes in their tumours and panel (B) depicts the group of patients with CRC with unknown *p53* status.

either directly or indirectly allow for the G-allele of SNP309 to accelerate tumour formation in women.[1] This model also predicts that women diagnosed with CRC when oestrogen levels are markedly greater (ie, women at a younger age or pre-menopausal age) should be enriched in the G-allele of SNP309 compared with patients diagnosed with CRC when oestrogen levels are markedly lower (ie, women at an older age or postmenopausal age, and men). Indeed, this can be seen in two independent groups of patients with CRC. Specifically, in the group of patients with CRC and wild-type *p53* genes in their tumours (fig 2A), patients with the G-allele of SNP309 make up 88% of the female patients with CRC diagnosed by the age of 55 years ($n = 17$), but only 60% of the women diagnosed above 55 years of age ($n = 50$, $p = 0.028$, Fisher's exact test) and 55% of the male patients with CRC ($n = 98$, $p = 0.008$, Fisher's exact test,). In a second group of patients with CRC, who were previously genotyped and the mutational status of the *p53* gene in the tumour was unknown[3] (fig 2B), patients with the G-allele of SNP309 make up 84% of the female patients with CRC diagnosed by the age of 55 years ($n = 75$), but only 67% of the female patients diagnosed above 55 years of age ($n = 406$, $p = 0.002$, Fisher's exact test) and 62% of the male patients with CRC ($n = 488$, $p<0.001$, Fisher's exact test,). Taken together, the data presented in this report lend significance to the observation that the G-allele of SNP309 accelerates colorectal tumour formation only in women and lend further support to the model that primarily female-specific hormones, such as oestrogen, could either directly or indirectly allow for the G-allele of SNP309 to accelerate tumour formation in women.[1]

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