LETTER TO JMG

# Tumour selection advantage of non-dominant negative *P53* mutations in homozygotic *MDM2*-SNP309 colorectal cancer cells

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**Background:** Mdm2 is a natural inhibitor of p53 function and its overexpression impairs p53 transcriptional activity. T→G single-nucleotide polymorphism at position 309 (SNP309) of mdm2 induces overexpression of mdm2, but inhibits p53.
**Objectives:** To determine whether SNP309 is a risk-modifier polymorphism in colorectal cancer (CRC) and whether tumour selection of *P53* mutations are influenced by SNP309.
**Methods:** Single-stranded conformation polymorphism and automatic sequencing were performed.
**Results:** SNP309 is not associated with the risk of CRC or recurrence of tumours. These data do not over-ride the tumour-selection capabilities of *P53* mutations in CRC. However, a significant association with non-dominant-negative *P53* mutations ($p = 0.02$) was found.
**Conclusions:** *MDM2*-SNP309 favours tumour selection of non-dominant negative *P53* mutations in CRC, which also show an earlier age of tumour onset.

Impairment of p53 function is a common feature in cancer cells and has been strongly associated with progression of cancer.[1–3] The transcriptional activity of p53 in response to DNA damage or cellular stress can lead to cell cycle arrest in normal cells, and to the activation of apoptotic and repair pathways.[1 4] As cell death or survival depends largely on the balance between apoptosis and repair, changes in p53 have become a central paradigm of tumorigenesis. Under normal homoeostatic conditions, cells show no p53-dependent transcriptional activity,[5] largely owing to the inhibitory effects of mdm2, a natural inhibitor of p53 that binds and targets p53 for ubiquitination and degradation.[4–6] Under conditions of stress, however, p53 levels increase and several downstream targets are trancriptionally activated.[5] In most tumours, inhibition of the p53 pathway is achieved by *P53* mutations or through the overexpression of mdm2.[3 7 8]

Approximately half of the colorectal cancers (CRCs) harbour mutations in *P53,* and overexpression of mdm2 can be found in one third of them, suggesting that the great majority of CRCs have a dysfunctional p53 pathway.[2 3 9] Further, it has been suggested that mdm2 might have a role in progression of colon cancer through both p53-dependent and p53-independent mechanisms, and a p53-independent role of mdm2 in mice tumorigenesis has also been reported.[10 11]

A T→G single-nucleotide polymorphism at position 309 (SNP309) of the promoter region of *MDM2* extends the length of an existing DNA binding site for the Sp1 transcription factor. This increases the affinity of Sp1 for the *MDM2* promoter and causes overexpression of mdm2. Accordingly, cells homozygotic for the SNP309 (GG) show the highest expression of mdm2 , whereas heterozygotic TG cells show intermediate levels of overexpression compared with the TT genotype.[12] In agreement with this, SNP309 has been shown to strongly diminish the activity of the p53 pathway.[13]

Recently, SNP309 was shown to be associated with development of tumours in patients with Li–Fraumeni syndrome, who have germline *P53* mutations. Interestingly, people with Li–Fraumeni syndrome with SNP309 with either homozygotic (GG) or heterozygotic (GT) status show early age of onset for several tumour types, suggesting that SNP309 is a potential cancer risk-modifier polymorphism.[12] Also, sporadic soft-tissue sarcomas from a selected cohort of patients show early age of tumour onset, suggesting that SNP309 does not require inactivating germline p53 mutations to increase susceptibility to cancer.[12]

However, the tumorigenic potential of *MDM2*-SNP309 in CRC (as well as in uterine leiomyosarcomas and squamous cell carcinoma of the head and neck) has been recently challenged,[14 15] questioning whether the incidence of SNP309 might contribute to risk of CRC and also whether SNP309 can modulate the positive selection exerted by *P53* mutations in tumour cells. In this study, we further investigate the possible contribution of *MDM2*-SNP309 to CRC using a series of 295 tumours. Moreover, to gain further insight into the apparent contradictory data in the literature, we studied the possible role of *MDM2*-SNP309 in CRC depending on *P53* mutations.

## MATERIALS AND METHODS

Tumours were obtained from the University Hospital Vall d'Hebron (Barcelona, Spain), the Catalan Institute of Oncology (Barcelona), the Sapporo Medical University (Sapporo, Japan), the Hospital of S Joao (Porto, Portugal) and also from several different hospitals in Finland. Accordingly, 61 tumours were obtained from Japan, 34 from Finland, 48 from Portugal and 152 from Spain. Control samples from healthy people >65 years of age and with no evidence of neoplastic diseases were also collected from the University Hospital Vall d'Hebron. Samples were collected in accordance with previously established ethical protocols from each one of the participating institutions, and the respective ethics committees approved the study. Genomic DNA was extracted with phenol–chloroform according to standard procedures. All tumours were analysed for the presence of microsatellite instability according to

**Abbreviations:** CRC, colorectal cancer; LOH, loss of heterozygosity; PCR, polymerase chain reaction; SNP309, single-nucleotide polymorphism at position 309; SSCP, single-stranded conformation polymorphism

international criteria, using various panels of dinucleotide and mononucleotide repeat sequences as described previously.[16] Only tumours negative for microsatellite instability were included in this study. A total of 295 tumours and 184 controls were analysed for the *MDM2* fragment encompassing nucleotide 309 by means of polymerase chain reaction single-stranded conformation polymorphism (PCR-SSCP) and sequencing. Primer sequences were 5′-CGG GAG TTC AGG GTA AAG GT-3′ and 5′-TCG GAA CGT GTC TGA ACT TG-3′. Genomic DNA (25–100 ng) was amplified by PCR with the addition of [$\alpha$-$^{32}$P] dCTP using the following cycling conditions: 30 s at 94℃, 30 s at 60℃ and 45 s at 72℃ for 35 cycles. PCR products were diluted with denaturing buffer (formamide with 0.025% xylene cyanol and 0.025% bromophenol blue) and heated up to 95℃ for 5 min before loading on to 0.8× mutation detection enhancement gels (Flowgen, Rockland, Maine, USA). Samples were run for 12–18 h and gels exposed to autoradiography. SSCP patterns for each genotype (TT, TG and GG) of SNP309 were previously characterised by sequencing analysis of representative cases on an ABI Prism 377 Automatic sequencer (Perkin-Elmer, Foster City, California, USA) using the ABI Prism Dye Terminator Cycle Sequencing Kit (Perkin-Elmer). The statuses of SNP309 in 40 of the analysed samples were previously reported.[14] Clinical follow-up of 5 years on average was available for 202 of the patients with CRC in this study. Data on *P53* mutations were already available in 260 cases. The relationship between the SNP309 genotype and the risk of CRC and *P53* mutations was assessed by odds ratio (OR) with 95% confidence interval (CI) limits. Significance ($p<0.05$) was assessed by the two-sided Fisher's exact test or the t test when adequate.

## RESULTS AND DISCUSSION

### Does *MDM2*-SNP309 predispose to CRC?

A total of 336 sporadic colorectal tumours and control samples from lymphocytes of healthy people >65 years of age from Spain were analysed for the SNP309. Genotypes TT, TG and GG were detected in 53%, 34% and 13% of control samples (n = 184) and in 44%, 45% and 11%, respectively, of colorectal tumours (n = 152; table 1). No association of the GG SNP309 with higher risk of CRC was detected (OR 1.04, 95% CI 0.52 to 2.08; p = 0.94), nor were differences found with heterozygotic and TT genotypes, suggesting that SNP309 is not a risk factor for sporadic colon cancer (table 1). Also no association was detected when considering tumours from Caucasian patients (Spanish and Portuguese; n = 200; not shown). Further, similar distributions of the T and G alleles were detected in tumours from Japan (n = 61) and Finland (n = 34).

Nonetheless, Bond *et al*[12] found, in people with Li–Fraumeni syndrome with monoallelic *P53* germline mutations and in sporadic soft-tissue sarcomas, that tumours from patients with the homozygotic GG genotype had early ages of tumour onset and higher frequencies of second tumours, suggesting that SNP309 might accelerate tumour progression and recurrence of tumours. An association of the GG genotype with early age of tumour onset has also been reported recently in CRCs with wild-type *P53* in a series of 153 patients.[17] In our Caucasian series, however, we found no differences among *MDM2*-SNP309 genotypes when the age of tumour onset was considered (p = 0.74). Also, we further studied whether SNP309 might be associated with recurrence of tumours and advanced stage of progression. For this purpose, we gathered a total of 261 CRC tumours from different origins as described previously. Risk assessment for early recurrence of tumours (<2 years from surgical retrieval of the tumours) and local versus metastatic disease (ie Dukes A/B *v* C/D stages) yielded an overall risk of recurrence of the G allele of 0.78 (95% CI 0.43 to 1.43; p = 0.44) and OR of 0.89 (95% CI 0.54 to 1.47; p = 0.75) for an advanced stage (table 2). No associations were detected when considering only the Caucasian series (not shown).

These results are in good agreement with a previous report showing that SNP309 made no significant contribution to formation of tumours.[14] Even though additional research is needed to investigate whether these observations also extend to other tumour types, SNP309, which has been shown to result in higher mdm2 levels and activity and can be found in approximately 13% of the population, does not seem to increase the risk of developing CRC.

### Does SNP309 over-ride the selective advantage imposed by *P53* mutations in colorectal tumours?

Although we found no association between SNP309 and risk and progression of CRC, we investigated the possibility that SNP309 could modulate the selective advantage imposed by *P53* mutations in colorectal tumours. We analysed the distribution of the SNP309 genotypes in 260 colon tumours with and without *P53* mutations.

Genotypes TT, TG and GG were detected in 46%, 42% and 12% of tumours with *P53* mutations (n = 134) and in 44%, 47% and 9%, respectively, of colorectal tumours with wild-type *P53* (n = 126). No differences were detected regarding the mutational status of *P53,* and a similar distribution of the SNP309 genotypes was found (p = 0.42). The OR for the GG genotype in the *P53* mutation group was 1.31 (95% CI 0.56 to 3.07; p = 0.67). We also studied whether tumours with or without *P53* mutations showed different ages of tumour onset regarding the SNP309 genotype. However, we found no differences (p = 0.75). Accordingly, our data show no evidence that SNP309 increases the susceptibility to colon cancer or alters the selection advantage of *P53* mutations in colorectal tumour cells.

### Does SNP309 favour the selection advantage of non-dominant negative *P53* mutations in colorectal tumours?

People with Li–Fraumeni syndrome inherit a mutated allele of *P53*. These people develop tumours significantly earlier if they have the G allele of SNP309 compared with patients with the TT allele. This is consistent with the idea that in these patients the G allele would result in hyperactive mdm2 and reduced p53

**Table 1** *MDM2*-single-nucleotide polymorphism at position 309 and risk of cancer in colorectal tumours from Spanish patients

| Genotype | Controls (n = 184) | CRC (n = 152) | OR | 95% CI | p Value |
|---|---|---|---|---|---|
| TT | 97 | 66 | 1.0 | | |
| TG | 63 | 69 | 1.61 | 1.01 to 2.55 | 0.06 |
| GG | 24 | 17 | 1.04 | 0.52 to 2.08 | 0.94 |
| TG/GG | 87 | 86 | 1.45 | 0.94 to 2.23 | 0.11 |

The total number of tumours analysed is indicated in parentheses.
CRC, colorectal cancer.

**Table 2** *MDM2*-single-nucleotide polymorphism at position 309, *P53* mutations and clinicopathological features in colorectal tumours

| Stage | Dukes A/B (n = 108) | Dukes C/D (n = 153) | OR | 95% CI | p Value |
|---|---|---|---|---|---|
| TT | 45 | 68 | 1.0 | | |
| TG | 51 | 66 | 0.85 | 0.50 to 1.45 | 0.59 |
| GG | 12 | 19 | 1.04 | 0.46 to 2.36 | 0.83 |
| TT/GG | 63 | 85 | 0.89 | 0.54–1.47 | 0.75 |
| Early recurrence | No (n = 114) | Yes (n = 50) | OR | 95% CI | p Value |
| Dukes B/C | | | | | |
| TT | 46 | 25 | 1.0 | | |
| TG | 56 | 21 | 0.69 | 0.34 to 1.38 | 0.29 |
| GG | 12 | 4 | 0.61 | 0.17 to 2.10 | 0.38 |
| TG/GG | 68 | 25 | 0.67 | 0.34 to 1.32 | 0.23 |
| Dukes C | (n = 56) | (n = 35) | | | |
| TT | 23 | 17 | 1.0 | | |
| TG | 26 | 15 | 0.78 | 0.32 to 1.90 | 0.65 |
| GG | 7 3 | 0.58 | 0.72 | 0.13 to 2.57 | |
| TG/GG | 33 | 18 | 0.74 | 0.31 to 1.73 | 0.52 |
| P53 mutations | Negative (n = 126) | Positive (n = 134) | OR | 95% CI | p Value |
| TT | 56 | 62 | 1.0 | | |
| TG | 59 | 56 | 0.85 | 0.51 to 1.43 | 0.60 |
| GG | 11 | 16 | 1.31 | 0.56 to 3.07 | 0.67 |
| TG/GG | 70 | 72 | 0.93 | 0.57 to 1.51 | 0.80 |
| P53 mutations | D-neg (n = 50) | Non-D-neg (n = 30) | OR | 95% CI | p Value |
| TT | 27 | 13 | 1.0 | | |
| TG | 21 | 10 | 0.99 | 0.36 to 2.69 | 0.79 |
| GG | 2 | 7 | 7.26 | 1.32 to 39.9 | **0.02*** |
| TG/GG | 23 | 17 | 1.53 | 0.61 to 3.82 | 0.48 |

The total number of tumours analysed is indicated in parentheses.
*Significance was achieved.
CRC, colorectal cancer; D-neg, tumours with dominant negative *P53* mutations; Non-D-neg, tumours with non-dominant-negative *P53* mutations.

activity. Nonetheless, in about half of the colorectal tumours from people with Li–Fraumeni syndrome, a second hit (mainly by loss of heterozygocity (LOH)) is required to achieve inhibition of the p53 pathway and tumour selection advantage.[18] Therefore, it is unlikely that SNP309 inactivates the p53 pathway as an underlying *P53* hit in these tumours, but instead might modulate tumour cell selection, together with *P53* mutations and LOH as a second hit. As stated earlier, we found no association of SNP309 with *P53* mutation status in our tumour series. However, as *P53* mutations show either dominant-negative or non-dominant-negative activity over the wild-type allele, we challenged the hypothesis that SNP309 could be a modulator of the selection-advantage capabilities of specific *P53* mutations. In this scenario, the incidence of non-dominant-negative mutations might be higher in colorectal tumours with a GG background, as this could downregulate the activity of the p53 pathway to levels that confer on tumour cells with *P53* mutations a growth advantage, even though complete inactivation of the p53 pathway might not be achieved until LOH of the wild-type *P53* allele occurs as a second hit.

In our series, the dominant-negative activity of *P53* mutations could be assigned to 80 of the analysed tumours according to experimental data retrieved from the *P53* Mutation Database of the International Agency for Research on Cancer (http://www-p53.iarc.fr/; table 3). Tumours were separated into two groups with dominant-negative (n = 50) and non-dominant-negative (n = 30) mutations.

We found a significant association between the GG genotype and the non-dominant-negative subgroup of tumours (OR 7.26, 95% CI 1.32 to 39.9, p = 0.021; table 2). Further, in the subset of tumours showing non-dominant-negative *P53* mutations, tumours with the GG genotype have a significantly earlier age of onset compared with tumours with the TT genotype (mean 61.1 (SD 6.6) *v* 73.1 (2.2) years, respectively; p = 0.048). No significant differences regarding age of tumour onset, Dukes stage or recurrence were assessed between groups of tumours classified according to the dominant-negative activity of *P53* mutations, although a clear trend for early recurrence of dominant-negative mutations was seen (p = 0.053; table 4). We therefore suggest that SNP309 might favour the selection of tumour cells with non-dominant-negative *P53* mutations.

In agreement with this hypothesis, dominant-negative *P53* mutations might sufficiently downregulate the p53 pathway to confer tumour cell selection, independently of the SNP309 status. It is noteworthy, however, that dominant-negative mutations are often not fully dominant, and therefore selection of tumour cells might also depend on the final modulation of the p53 pathway exerted by *P53* mutations, LOH or the presence of GG SNP309. Indeed, although GG SNP309 associates with non-dominant-negative *P53* mutations, some cases with dominant-negative mutations also showed GG SNP309. Further, LOH of *P53* was also detected in some of the analysed tumours, independently of the SNP309 genotype and the dominant-negative activity of *P53* mutations (not shown). Nonetheless, dominant-negative *P53* mutations occur with the same frequency in people with the TT, TG or GG SNP309 genotype. In this context, acquisition of a monoallelic non-dominant-negative *P53* mutation in intestinal cells from people with a GG SNP309 would be sufficient to confer on these cells a growth advantage and favour progression of tumours. In this scenario, the presence of GG SNP309 might decrease the levels of p53, compromising the functionality of the p53 pathway and conferring advantage on the tumour cell. Whether this selection is motivated by SNP309 itself or due to a limited cellular response to oncogenic stress signals is still to be investigated. It is unlikely, however, that SNP309 is equivalent to a second hit as LOH. Most likely, non-dominant-negative *P53* mutations in cells from people with a TT SNP309 would not confer enough selective advantage, and a second hit would be required in these people to promote tumour cell progression. This might

**Table 3** Dominant-negative activity of *P53* mutations in the tumours

| Tumour | Patient age (years) | Dukes stage | Recurrence* | *P53* status | 309 | D-neg activity† | AA change |
|---|---|---|---|---|---|---|---|
| 17 | 65 | B | NA | Mut | G | Negative | Arg282Trp |
| 113 | 73 | B | 1 | Mut | T | Negative | Arg282Trp |
| 130 | 75 | B | 0 | Mut | T | Negative | Arg231Stop |
| 142 | 91 | B | 0 | Mut | T | Negative | Arg231Stop |
| 300 | 73 | B | 0 | Mut | T | Negative | Arg282Trp |
| 109 | 43 | B | 0 | Mut | TG | Negative | Gly266Val |
| 14 | 70 | B | NA | Mut | TG | Negative | Arg196Stop |
| 15 | 58 | B | NA | Mut | TG | Negative | Arg213Stop |
| 38 | 58 | B | 0 | Mut | TG | Negative | Gly266Arg |
| 127 | 31 | C | 0 | Mut | G | Negative | Gly266Arg |
| 595 | 58 | C | 0 | Mut | G | Negative | Cys275Tyr |
| 63 | 74 | C | 0 | Mut | T | Negative | Pro152Leu |
| 190 | 58 | C | 0 | Mut | T | Negative | Tyr220Cys |
| 282 | 78 | C | 1 | Mut | T | Negative | Arg282Trp |
| 323 | 65 | C | 0 | Mut | T | Negative | Arg158Cys |
| 917 | 72 | C | 0 | Mut | T | Negative | Ile195Thr |
| 123 | 70 | C | 1 | Mut | T | Negative | Gly266Val |
| 238 | 72 | C | 0 | Mut | TG | Negative | Arg282Trp |
| 269 | 81 | C | 0 | Mut | TG | Negative | rg282Trp |
| 11880/01 | 62 | C | NA | Mut | TG | Negative | Gly266Arg |
| 71 | 55 | D | 1 | Mut | G | Negative | Arg282Trp |
| 304 | 65 | D | 0 | Mut | G | Negative | Arg282Trp |
| 4560/02 | 90 | D | NA | Mut | G | Negative | Tyr220His |
| 107 | 79 | D | 0 | Mut | T | Negative | Arg282Trp |
| 121 | 77 | D | 0 | Mut | T | Negative | Arg158His |
| 1013/03 | 65 | D | NA | Mut | T | Negative | Gly262Asp |
| 132 | 54 | D | 0 | Mut | TG | Negative | Arg196Stop |
| 28 | 57 | D | 1 | Mut | TG | Negative | Ser215Asn |
| 452/02 | 56 | NA | NA | Mut | TG | Negative | Arg282Trp |
| 939 | 64 | C | 0 | Mut | G | Negative | Arg282Trp |
| 20 | 76 | A | NA | Mut | T | Positive | Arg175His |
| 25 | 54 | A | 0 | Mut | TG | Positive | Arg175His |
| 27 | 62 | A | 0 | Mut | TG | Positive | Arg248Gln |
| 31 | 65 | A | 0 | Mut | TG | Positive | Gly245Asp |
| 138 | 80 | B | 0 | Mut | T | Positive | Gly245Asp |
| 145 | 73 | B | 0 | Mut | T | Positive | Arg175His |
| 12 | 78 | B | NA | Mut | T | Positive | Arg175His |
| 18 | 68 | B | NA | Mut | T | Positive | Arg175His |
| 19 | 86 | B | NA | Mut | T | Positive | Arg175His |
| 39 | 79 | B | 1 | Mut | T | Positive | Arg273Cys |
| 40 | 60 | B | 1 | Mut | T | Positive | Arg248Gln |
| 3 | 76 | B | 0 | Mut | TG | Positive | Arg248Trp |
| 52 | 70 | B | 0 | Mut | TG | Positive | Arg273His |
| 75 | 77 | B | 1 | Mut | TG | Positive | Pro250Leu |
| 151 | 79 | B | 1 | Mut | TG | Positive | Gly245Asp |
| 239 | 74 | B | 0 | Mut | TG | Positive | Arg273Cys |
| 29 | 65 | B | 0 | Mut | TG | Positive | Arg248Trp |
| 16 | 70 | C | 0 | Mut | T | Positive | Asp184Tyr |
| 26 | 64 | C | 0 | Mut | T | Positive | Ser241Thr |
| 72 | 73 | C | 1 | Mut | T | Positive | Gly245Asp |
| 139 | 49 | C | 1 | Mut | T | Positive | Arg175His |
| 226 | 55 | C | 0 | Mut | T | Positive | Arg273Cys |
| 13 | 59 | C | NA | Mut | T | Positive | Arg175His |
| 23 | 69 | C | 0 | Mut | T | Positive | Arg248Glu |
| 17 | 31 | C | 0 | Mut | TG | Positive | Arg248Trp |
| 104 | 61 | C | 1 | Mut | TG | Positive | Lys132Asn |
| 164 | 57 | C | 0 | Mut | TG | Positive | Arg273Cys |
| 165 | 76 | C | 1 | Mut | TG | Positive | Arg248Trp |
| 11 | 45 | C | NA | Mut | TG | Positive | Arg175His |
| 37 | 75 | C | 0 | Mut | TG | Positive | Arg273Cys |
| 664 | 72 | C | 1 | Mut | TG | Positive | Arg273His |
| 931 | 63 | C | 1 | Mut | TG | Positive | Gly245Ser |
| 981 | 56 | C | 1 | Mut | TG | Positive | Arg273His |
| 199 | 84 | D | 0 | Mut | G | Positive | Arg175His |
| 41 | 61 | D | NA | Mut | G | Positive | Arg175His |
| 101 | 48 | D | 1 | Mut | T | Positive | Arg175His |
| 986/03 | 68 | D | NA | Mut | T | Positive | Gly245Ser |
| 4561/02 | NA | D | NA | Mut | T | Positive | Arg248Trp |
| 989/03 | 46 | D | NA | Mut | TG | Positive | Arg273Leu |
| 920/03 | 80 | NA | NA | Mut | T | Positive | Cys117Arg |
| 929/03 | 73 | NA | NA | Mut | T | Positive | Gly245Ser |
| 454/02 | 70 | NA | NA | Mut | T | Positive | Arg175His |
| 938/03 | 67 | NA | NA | Mut | TG | Positive | Cys176Phe |
| 5574/01 | 80 | NA | NA | Mut | TG | Positive | Arg175His |
| 24 | 89 | B | NA | Mut | T | Positive | Arg273His |
| 23B | 67 | C | 1 | Mut | T | Positive | Gly245Asp |
| 26 | 64 | C | 1 | Mut | T | Positive | Arg248Gln |
| 33 | 79 | C | 0 | Mut | T | Positive | Arg175His |

**Table 3** Continued

| Tumour | Patient age (years) | Dukes stage | Recurrence* | *P53* status | 309 | D-neg activity† | AA change |
|---|---|---|---|---|---|---|---|
| 35 | 61 | C | 1 | Mut | T | Positive | Arg248Gln |
| 22 | 76 | D | 1 | Mut | T | Positive | Arg175His |

*Early recurrence (<2 years from intervention) is indicated as positive (1) or negative (0).
†Dominant-negative activity for the *P53* mutation is indicated (experimental data available at the International Agency for Research on Cancer-P53 Mutation Database http://www-p53.iarc.fr/).
Mut, mutation; NA, not available.

**Table 4** Clinicopathological features of *P53* mutations and dominant-negative activity

| | Dominant-negative activity | | |
|---|---|---|---|
| | Positive | Negative | p Value |
| Mean (SD) age in years | 67.55 (11.73) | 66.3 (12.70) | 0.66 |
| Stage | | | 0.47 |
| Dukes A/B | 18 | 9 | |
| Dukes C/D | 27 | 20 | |
| Recurrence | | | 0.053 |
| Positive | 16 | 5 | |
| Negative | 17 | 18 | |

explain the significantly younger age of tumour onset in patients with a GG genotype. These results, therefore, may help to explain the apparently contradictive data regarding SNP309 and cancer incidence, and also question whether data from previous p53-association studies might need to be re-evaluated according to the dominant-negative activity of *P53* mutations.


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## Key points

- *MDM2*-single-nulceotide polymorphism at position 309 (SNP309) is not a risk-modifier polymorphism of human colorectal cancer and therefore is not associated with the incidence of colon cancer.
- *MDM2*-SNP309 is not associated with dominant-negative *P53* mutations in colon tumours or with tumour recurrence.
- *MDM2*-SNP309, however, is associated with non-dominant-negative *P53* mutations in a specific subset of tumours, which also show a significantly earlier age of tumour onset, suggesting that it might favour tumour selection of P53 mutations with non-dominant-negative activity.


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