IJC
International Journal of Cancer

Influence of *MDM2* SNP309 and SNP285 status on the risk of cancer in the breast, prostate, lung and colon

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MDM2 is a key regulator of the p53 tumor suppressor protein and is overexpressed in many human cancers. Two single nucleotide polymorphisms (SNPs) located in the *MDM2* intronic promoter (P2) have been found to exert biological function. The G-allele of SNP309T>G; rs2279744 increases *MDM2* transcription and has been linked to increased cancer risk. In contrast, the less frequent SNP285G>C; rs117039649, which is in complete linkage disequilibrium with SNP309 (generating a SNP285C/309G variant haplotype), has been related to reduced *MDM2* transcription and to reduced risk of breast, endometrial and ovarian cancer. In this large population-based case–control study, we genotyped SNP309 and SNP285 in 10,830 individuals, including cases with cancer of the breast (n=1,717), colon (n=1,532), lung (n=1,331) and prostate (n=2,501), as well as 3,749 non-cancer controls. We found a slightly reduced risk for lung cancer among individuals harboring the SNP309TG/GG genotypes compared to the SNP309TT genotype (OR= 0.86; CI = 0.67–0.98), but this association was restricted to women (OR = 0.77; CI = 0.63–0.95) and was not present among men (OR = 0.91; CI = 0.77–1.08). Consistent with previous findings, we found a reduced risk for breast cancer among individuals carrying the SNP285GC/309GG genotype versus the SNP285GG/309GG genotype (OR = 0.55; CI = 0.33–0.93). In conclusion, our data support the hypothesis that the effects of both SNP285 and SNP309 status are tissue dependent.

Cancer Genetics

The protein product of the human homologue of Murine Double Minute 2 (MDM2) gene is a key regulator of the p53 tumor suppressor protein. The negative regulation is primarily executed through MDM2's function as an ubiquitin E3 ligase,[1,2] but MDM2 also inhibits the transcriptional activity of p53 through direct binding.[3,4] Recently, Gaijjer et al.[5] reported the MDM2 protein to bind and stabilize p53 mRNA in response to genotoxic stress, thereby adding further evidence to this complex interaction. Since *MDM2* harbors p53 responsive elements in its inducible intronic promoter P2, a fine tuned negative feedback loop mechanism is likely to be present.[6]

Elevated MDM2 levels due to *MDM2* gene amplification and/or protein overexpression has been observed in several human cancers harboring wild-type p53,[7,8] and has been suggested to be an alternative mechanism for p53 inactivation in tumorigenesis and tumor progression.[9]

A functional single nucleotide polymorphism (SNP) located at position 309 downstream of exon 1 (SNP309 T>G; rs2279744) within the *MDM2* intronic P2 promoter has been shown to enhance binding of the Sp1 transcription factor, resulting in elevated *MDM2* expression levels.[10] The initial reports indicated the SNP309G variant to be associated with increased cancer risk and early cancer development.[10–12] Subsequently, epidemiological studies covering several cancer forms and different ethnic groups have assessed the impact of SNP309G on tumor risk and age at diagnosis.[13–15] Taken together, the data from these studies indicate that SNP309G increases cancer risk among individuals of Asian ancestry, while the results in Europeans have been inconsistent.[13,14]

Recently, we reported a second polymorphism in the promoter P2 region of *MDM2*, (SNP285 G>C; rs117039649) situated 24 bps upstream of SNP309.[16] The SNP285C variant resides on the SNP309G allele, thus forming a distinct

Key words: MDM2, SNP285, SNP309, cancer risk

Abbreviations: CI: confidence interval; CONOR: cohort of Norway; ERE: estrogen receptor binding element; ESR1: estrogen receptor 1; OR: odds ratio; REK Midt-Norge: Regional Committee for Ethics in Medical Research; SNP: single nucleotide polymorphism; Sp1: specificity protein 1.

Additional Supporting Information may be found in the online version of this article.

Grant sponsors: Norwegian Cancer Society, the Norwegian Health Region West funding body and the Bergen Medical Research Foundation

DOI: 10.1002/ijc.29358

History: Received 20 Oct 2014; Accepted 6 Nov 2014; Online 27 Nov 2014

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What's new?

The protein MDM2 grabs on to the tumor suppressor p53, stopping its transcription activity. In this study, the authors investigated two MDM2 polymorphisms. The first, SNP309G, has been linked to increased cancer risk; the more recently identified SNP285C may modulate the effects SNP309G. After screening 7,000 cases and half as many controls, the authors found a tissue-specific effect. They report that SNP285C accompanies reduced breast cancer risk among those carrying two SNP309G alleles. No association of SNP309 allele with colon, breast, or prostate cancer risk could be detected, but they noted an association of SNP309G with reduced lung cancer risk.

SNP285C/309G haplotype.[16,17] The C-allele of SNP285 seems to antagonize the effect of SNP309G by reducing the binding affinity of the Sp1 transcription factor to the *MDM2* promoter.[16,18] Moreover, the SNP285C/309G haplotype has been associated with a reduced risk for ovarian, breast and endometrial cancer[16,19,20] but was found not to be associated with risk for cancer of the prostate or lung.[19,21] Finally, the SNP285C has also been found to reduce the risk of *BRCA1* related ovarian cancer in individuals carrying the SNP309G allele.[22]

While the SNP285C/309G haplotype is observed in approximately 11% of the SNP309G alleles across all Caucasian populations, except for a low frequency in the Finnish and the Saami populations (1.9% and 0.3%, respectively), it is absent among individuals of Asian (Chinese, Mongolian and Japanese) and African (Afro-Americans, Kenyans and Nigerians) heritage.[16,23,24] Thus, the presence of the SNP285C variant in Europeans could be an underlying reason for the conflicting findings regarding SNP309G on cancer risk in different ethnic groups.

Considering the fact that the SNP285C has been found to reduce the risk of female cancers (ovarian, endometrial and breast) but not cancer of the prostate,[16,19,20] it is interesting to note that the estrogen receptor (ESR1) has been reported to act as a transcriptional activator in concert with Sp1[25,26] and *in silico* predictions have shown that the *MDM2* P2 promoter contains an estrogen receptor binding element (ERE) overlapping the Sp1 binding site harboring SNP285.[19] Taken together, this may suggest that the SNP285C allele could have a gender-specific cancer protective effect.

To assess potential effects of SNP309G and SNP285C on cancer risk at a broader scale, we here conducted a large scale screening of *MDM2* SNP285 and SNP309 status in a population based case–control study in Norway. Thus, we included a total of 7,081 cancer incident cases (breast, prostate, colon and lung), and 3,479 non-cancer controls. We confirmed our previous findings that SNP285C may act as a protective factor against cancer of the breast but not against prostate cancer. Further, we found a moderate positive association between SNP309TT status as well as SNP285C and lung cancer risk in women, but found no effect of these two SNP variants and lung cancer risk in men; neither did we observe any association with colon cancer risk in either gender. Our findings argue against a major effect of SNP309 status on the risk for cancer of the breast, prostate, lung or colon. In contrast, they indicate a possible tissue specific protective effect of SNP285C on cancer risk.

Material and Methods

Study Population

All cases and control samples in this study were obtained from the population-based Cohort of Norway (CONOR) study.[27] All incident cancers were identified by linking the identity of individuals participating in the CONOR study to the Norwegian Cancer Registry from entry until the end of 2010. Thus, we analyzed 1,717 incident cases of breast cancer, 1,532 colon cancers, 1,331 lung cancers and 2,501 prostate cancers (for details, see Supporting Information Fig. 1). As a reference group, a total of 3,749 healthy controls from the same cohort (matched to the cases with respect to age (in five years groups), area of residence and gender) were analyzed (Supporting Information Fig. 1).

Six hundred and five of the prostate cancer cases were included in a previous study.[19] In addition, 71 of the male controls included in this study overlapped with the controls used in our previous study,[19] thus, our study represents an extension of the previous analysis related to prostate cancer.

With respect to smoking status, information about smoking (current, former, never) was available for 1,264 lung cancer cases and 3,429 controls.

All sample donors included in the study had provided written informed consent to anonymous genetic testing for scientific purposes, and the study was approved by the Regional Committee for Ethics in Medical Research (REK Midt-Norge).

MDM2 Promoter Screening

All samples were genotyped for *MDM2* SNP285 (rs117039649) and SNP309 (rs2279744) using custom LightSNiP assays (TIB MOLBIOL Syntheselabor GmbH, Berlin, Germany) on a LightCycler 480 II instrument (Roche, Basel, Switzerland) as previously described in detail elsewhere.[24]

Statistical Analysis

Potential deviations from Hardy-Weinberg equilibrium were assessed by calculating the expected genotype distribution based on the observed allele frequencies and compared with the observed genotype distribution using Chi-square tests.

Potential associations between *MDM2* SNP309 and SNP285 and risk for cancer of the breast, colon, lung and prostate were estimated by calculating odds ratio (OR) with 95% confidence intervals (CI). The analyses were stratified by

Table 1. *MDM2* SNP309 distribution and cancer risk

Cases/controls	Genotype SNP309 *n* (%)			OR (95% CI) SNP309 GG+TG vs. TT	Fisher exact	OR (95% CI) SNP309 GG vs. TG+TT	Fisher exact
	TT	TG	GG				
Controls	1464 (39.1)	1783 (47.6)	502 (13.4)	1.00	–	1.00	–
Women	740 (39.5)	878 (46.9)	254 (16.6)	1.00	–	1.00	–
Men	724 (38.6)	905 (48.2)	248 (13.2)	1.00	–	1.00	–
Controls[1]	1464 (41.9)	1615 (46.2)	416 (11.9)	1.00	–	1.00	–
Colon cancer	631 (41.2)	715 (46.7)	186 (12.1)	0.92 (0.81–1.03)	0.154	0.89 (0.75–1.07)	0.224
Women[2]	322 (41.3)	363 (46.6)	94 (12.1)	0.96 (0.81–1.15)	0.685	0.91 (0.70–1.19)	0.496
Men[2]	309 (41.0)	352 (46.8)	92 (12.2)	0.91 (0.77–1.10)	0.317	0.87 (0.67–1.13)	0.289
Colon cancer[1]	631 (44.1)	642 (44.9)	157 (11.0)	0.91 (0.81–1.03)	0.153	0.91 (0.75–1.11)	0.378
Lung cancer	567 (42.6)	581 (43.7)	183 (13.8)	0.86 (0.76–0.98)	0.025	1.03 (0.86–1.24)	0.744
Women[2]	228 (45.9)	203 (40.9)	66 (13.3)	0.77 (0.63–0.95)	0.014	0.91 (0.77–1.08)	0.278
Men[2]	339 (40.7)	378 (45.3)	117 (14.0)	0.91 (0.77–1.08)	0.278	1.03 (0.81–1.31)	0.790
Lung cancer[1]	567 (46.0)	520 (42.2)	145 (11.8)	0.85 (0.74–0.96)	0.012	0.99 (0.81–1.21)	0.918
Breast cancer	672 (39.1)	794 (46.2)	251 (14.6)	1.00 (0.89–1.12)	0.952	1.11 (0.94–1.30)	0.221
Women[2,3]	672 (39.1)	794 (46.2)	251 (14.6)	1.01 (0.88–1.16)	0.898	1.08 (0.98–1.31)	0.459
Breast cancer[1]	672 (41.6)	723 (44.8)	219 (13.6)	1.01 (0.90–1.14)	0.879	1.16 (0.89–1.39)	0.101
Prostate cancer	988 (39.5)	1169 (46.7)	344 (13.8)	0.98 (0.89–1.09)	0.731	1.03 (0.89–1.20)	0.678
Men[2,4]	988 (39.5)	1169 (46.7)	344 (13.8)	0.97 (0.86–1.10)	0.603	1.02 (0.86–1.22)	0.791
Prostate cancer[1]	988 (42.6)	1063 (45.8)	268 (11.6)	0.97 (0.87–1.08)	0.606	0.97 (0.82–1.14)	0.708

[1]Calculations without individuals carrying SNP285C.
[2]Age and population adjusted.
[3]Calculations against female controls only.
[4]Calculations against male controls only.

sex and in multivariable analyses we adjusted for the matching variables age and area of residence.

Statistical analyses were performed using the IBM SPSS statistics (version 19) software package and stata 13.0 for windows.

Results

Distribution of *MDM2* SNP285 and SNP309

In this study 10,830 Norwegian individuals (7,081 cancer cases and 3,749 healthy controls) were analyzed for *MDM2* SNP285 and SNP309 status. Among the healthy controls, we observed the minor allele frequencies (MAF) of SNP285 and SNP309 to be 0.034 and 0.372 respectively. This is in accordance with previous findings in both Norwegians and Europeans in general.[23,24,28] The genotype distribution was found to be in Hardy-Weinberg equilibrium for both SNPs ($p > 0.126$). No gender differences regarding distribution of any of the haplotypes were recorded (Chi-square; $p = 0.723$ and $p = 0.530$ for SNP309 and SNP285 respectively), thus the association analyses (estimating ORs) were performed both by treating healthy controls as one homogenous group and after stratification according to gender.

Similar to our previous analyses,[16,19,24] we observed the SNP285C variant exclusively in individuals harboring the SNP309TG or GG genotype, confirming SNP285C to reside on a distinct SNP285C/309G haplotype.

Association Between *MDM2* SNP309 and Cancer Risk

A comprehensive overview of the SNP309 distribution in the four cancer types analyzed is given in Table 1. Using the dominant model (SNP309GG+TG versus TT), we observed no significant association between SNP309 status and risk of either breast, prostate or colon cancer. Recalculating the risk estimates adjusting for age and gender did not change the conclusions for either of the cancer types (Table 1).

Interestingly, we observed a negative association with lung cancer risk for SNP309G carriers (dominant model, SNP309TG+GG versus SNP309TT; Crude OR = 0.86; 95% CI = 0.76–0.98; Table 1). Further stratification suggested that the negative association was present in individuals harboring the SNP309TG genotype (Crude OR = 0.84; 95% CI = 0.74–0.96), but not for those harboring the SNP309GG genotype (Crude OR = 0.94; 95% CI = 0.77–1.14; Supporting Information Table 1). Notably, SNP309 status was associated with a reduced risk of lung cancer in women (age and population adjusted OR = 0.77; 95% CI = 0.63–0.95), but not in men (age and population adjusted OR = 0.91; 95% CI = 0.77–1.08).

Most studies addressing the effect of SNP309 status on cancer risk have not included information on SNP285 status in their analysis. In order to determine a potential confounding

Table 2. Effect of *MDM2* SNP285 on breast, colon, lung, and prostate cancer

	Genotype SNP285 *n* (%)			OR (95% CI) SNP285	
Cases/controls	GG	GC	CC	CC+GC vs. GG	Fisher exact
Controls	3495 (93.2)	254 (6.8)	0 (0.0)	1.00	–
Women	1750 (93.5)	122 (6.5)	0 (0.0)	1.00	–
Men	1745 (93.0)	132 (7.0)	0 (0.0)	1.00	–
Colon cancer	1430 (93.3)	99 (6.5)	3 (0.2)	0.98 (0.77–1.25)	0.904
Women[1]	720 (92.4)	57 (7.3)	2 (0.3)	1.15 (0.82–1.61)	0.412
Men[1]	710 (94.3)	42 (5.6)	1 (0.1)	0.77 (0.54–1.12)	0.166
Lung cancer	1232 (92.6)	98 (7.4)	1 (0.1)	1.11 (0.87–1.41)	0.415
Women[1]	457 (92.0)	39 (7.9)	1 (0.2)	1.21 (0.83–1.77)	0.326
Men[1]	775 (93.0)	59 (7.1)	0 (0.0)	1.03 (0.74–1.42)	0.880
Breast cancer	1614 (94.0)	100 (5.8)	3 (0.2)	0.88 (0.69–1.11)	0.289
Women[1,2]	1614 (94.0)	100 (5.8)	3 (0.2)	0.89 (0.67–1.17)	0.395
Prostate cancer	2319 (92.7)	175 (7.0)	7 (0.3)	1.08 (0.89–1.32)	0.448
Men[1,3]	2319 (92.7)	175 (7.0)	7 (0.3)	1.01 (0.80–1.28)	0.931

[1]Age and population adjusted.
[2]Calculations against female controls only.
[3]Calculations against male controls only.

effect of SNP285C, we reanalyzed the dataset after excluding individuals carrying the minor SNP285C allele from both cases and controls, but the estimated associations (ORs) remained essentially unchanged (Table 1).

Since 94.5% of the lung cancer patients (from whom we had data) were smokers, no formal assessment of a potential interaction between smoking status, *MDM2* SNP status and lung cancer risk could be performed. Excluding non-smokers from the analysis had little impact on the estimates (Supporting Information Table 1).

Association Between *MDM2* SNP285 Status and Cancer Risk

The proportion of SNP285CC carriers was 0.13% only; thus, we assessed the potential impact of SNP285 on cancer risk using the dominant model (genotypes CC+GC combined versus GG). Overall assessment (testing for OR related to SNP285 status with no stratification for SNP309 status, and with age, gender and population adjustments) showed no clear associations between *MDM2* SNP285C status and cancer risk for any of the cancer forms (Table 2/Fig. 1*a*).

Since the SNP285C allele resides on the SNP309G allele, similar to what was done in our previous studies[16,19]; we refined the analyses by removing individuals harboring the SNP309TT genotype, and stratified the data into the two subgroups of individuals harboring the SNP309TG - and GG genotypes. Interestingly, this subgroup analysis showed a negative association of SNP285C with breast cancer among individuals carrying the SNP309GG genotype (OR = 0.71; 95% CI =0.46–1.10; Table 3/Fig. 1*b*), but not among carriers of the SNP309TG genotype (OR = 0.94; CI 0.71–1.26; Table 4/Fig. 1*c*). Both these observations are in line with our previous finding suggesting that SNP285C is negatively associated with breast cancer risk among individuals homozygous for SNP309GG but not among 309TG heterozygotes.[16] Notably, restricting the analysis to individuals from the same geographical regions of Norway as our previous study[16] we observed an even stronger negative association of SNP285C with breast cancer risk in individuals harboring the SNP309GG/285GC genotype (OR = 0.51; 95% CI = 0.31–0.85; Supporting Information Table 2). Adjusting the estimated OR for age, study population and gender gave similar results (OR = 0.55; CI = 0.55; 95% CI = 0.33–0.93; Table 3/Fig. 1*b*).

In the SNP309 stratified analysis, no effect of SNP285C was observed on prostate cancer risk (Tables 3 and 4, Figs. 1*b* and 1*c*), confirming our previous findings from a smaller study.[19] Among individuals harboring the SNP309TG genotype, we found that SNP285C was positively associated with lung cancer risk in women (age and population adjusted OR = 1.64; 95% CI = 1.01–2.66) but not in men (age and population adjusted OR = 0.86; 95% CI = 0.56–1.32) (Table 4). Regarding cancer of the colon, we observed no clear effect of SNP285 status among carriers of either SNP309TG or the SNP309GG genotype among either males or females (Tables 3 and 4); nor did we observe any differences when tumors of the right and the left side of the colon were analyzed for separately (Supporting Information Table 3).

Discussion

While SNP309G was initially found to accelerate tumor formation in a hormone dependent manner and to be associated

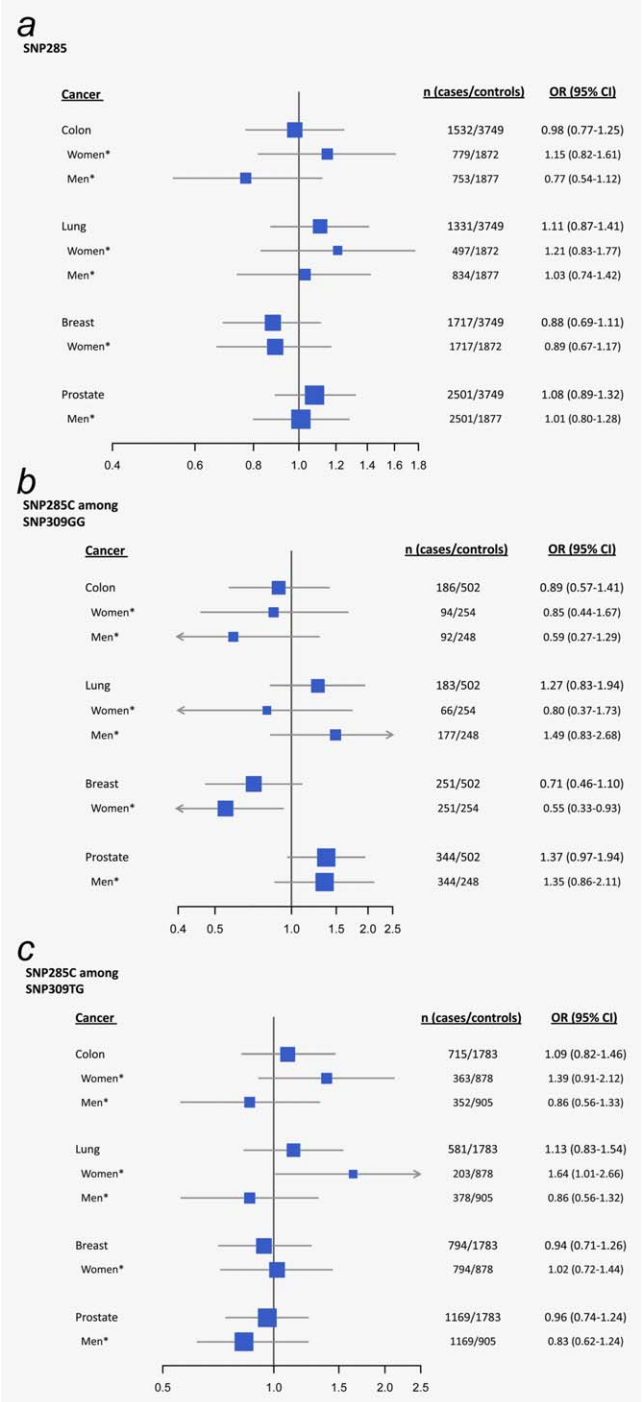

Figure 1. Impact of SNP285 on cancer risk. Forest plots showing the effect of SNP285 on colon, lung, breast and prostatic cancer, as compared to healthy controls, among the total study population (*a*), among individuals harboring the SNP309GG genotype (*b*) and the SNP309TG genotype (*c*). ORs were calculated as crude ORs for each cancer type. In addition, ORs were calculated using gender matched controls only, with adjustments for age and population (*).

with an increased risk of several malignancies,[11,12,29] subsequent case control studies examining the OR for different cancer forms across different ethnic populations have yielded conflicting results.[13–15,30–34] Taken together, much evidence points to a difference related to ethnicity, in as much as most individual studies have reported a positive association between the SNP309G genotype and tumor risk in Asians but not in Europeans.[13,14] Notably, most individual reports have enrolled a limited number of participants in their studies. In addition, the case control design of many studies may imply potential biased comparisons between patient and control groups, and a publication bias in favor of studies reporting positive results cannot be excluded.

There are several strengths of our study. First, the number of participants is high compared to other studies that have addressed *MDM2* SNPs and cancer risk. Second, this is a population-based study, where incident cancer cases and controls without cancer are drawn from the same underlying population. Third, the controls were matched to the cases with respect to sex, age and county of residence.

In this study, we found no association between *MDM2* SNP309 genotypes and the risk of either colon-, breast- or prostate cancer. While cancer of the left and the right side of the colon may differ according to age at diagnosis and genetic disposition,[35] subgroup analyses indicated no association between either of the two *MDM2* SNPs and the risk for either cancer of the left or the right side of the colon. Our observations regarding breast and colon cancer risk with respect to SNP309T/G status are in line with previous reports,[16,28,36] suggesting that the SNP309G allele may increase the risk in Asian populations[37] but not in Caucasians[14,15] Further, our finding that SNP309 status was not associated with risk for prostate cancer is in line with our previous finding in a smaller Norwegian study where some prostate cancer cases, but only 1.9% the controls, overlapped with the cases of our study.[19] The finding, however, differs from the results by Liu et al., who reported that the SNP309G allele may be negatively associated with prostate cancer risk in Caucasians.[31] Notably, the number of individuals analyzed in our study (2,501 prostate cancer patients and 3,749 healthy controls) are substantially higher than the number of individuals enrolled in the study by Liu et al (872 prostate cancer patients and 1,005 healthy controls), raising the possibility that the findings by Liu et al[31] may have occurred by chance.

Our finding that the SNP309G allele was associated with a reduced risk for lung cancer in individuals harboring the SNP309TG genotype differs from the findings of another Norwegian study which reported that carriers of the SNP309GG genotype may be at increased risk of lung cancer.[38] However, that study included a limited number of individuals (341 cases and 412 controls). In contrast, our present findings are in line with the observations of Li et al. who conducted a case–control study of 1,026 cases and 1,145 controls.[39] Notably, they reported a reduced risk among women, but not among men, indicating a possible gender difference with respect to SNP309 status and lung cancer risk. Interestingly, we observed that the SNP309G-allele was negatively associated with lung cancer risk among individuals carrying the SNP309TG but not

Table 3. Effect of *MDM2* SNP285C among SNP309GG

Cases/controls	Genotype SNP285 *n* (%) GG	GC	CC	OR (95% CI) SNP285 CC+GC vs. GG	Fisher exact
Controls	416 (82.9)	86 (17.1)	0 (0.0)	1.00	–
Women	208 (81.9)	46 (18.1)	0 (0.0)	1.00	–
Men	208 (83.9)	40 (16.1)	0 (0.0)	1.00	–
Colon cancer	157 (84.4)	26 (14.0)	3 (1.6)	0.89 (0.57–1.41)	0.730
Women[1]	76 (80.9)	16 (17.0)	2 (2.1)	0.85 (0.44–1.67)	0.541
Men[1]	81 (88.0)	10 (10.9)	1 (1.1)	0.59 (0.27–1.29)	0.186
Lung cancer	145 (79.2)	37 (20.2)	1 (0.6)	1.27 (0.83–1.94)	0.313
Women[1]	53 (80.3)	12 (18.2)	1 (1.5)	0.80 (0.37–1.73)	0.571
Men[1]	92 (78.6)	25 (21.4)	0 (0.0)	1.49 (0.83–2.68)	0.178
Breast cancer	219 (87.3)	29 (11.6)	3 (1.2)	0.71 (0.46–1.10)	0.137
Women[1,2]	219 (87.3)	29 (11.6)	3 (1.2)	0.55 (0.33–0.93)	0.027
Prostate cancer	268 (77.9)	69 (20.1)	7 (2.0)	1.37 (0.97–1.94)	0.076
Men[1,3]	268 (77.9)	69 (20.1)	7 (2.0)	1.35 (0.86–2.11)	0.187

[1]Age and population adjusted.
[2]Calculations against female controls only.
[3]Calculations against male controls only.

Table 4. Effect of *MDM2* SNP285C among SNP309TG

Cases/controls	Genotype SNP285 *n* (%) GG	GC	CC	OR (95% CI) SNP285 GC vs. GG	Fisher exact
Controls	1615 (90.6)	168 (9.4)	–	1.00	–
Women	802 (91.3)	76 (8.7)	–	1.00	–
Men	813 (89.8)	92 (10.2)	–	1.00	–
Colon cancer	642 (89.8)	73 (10.2)	–	1.09 (0.82–1.46)	0.549
Women[1]	322 (88.7)	41 (11.3)	–	1.39 (0.91–2.12)	0.131
Men[1]	320 (90.9)	32 (9.1)	–	0.86 (0.56–1.33)	0.495
Lung cancer	520 (89.5)	61 (10.5)	–	1.13 (0.83–1.54)	0.467
Women[1]	176 (86.7)	27 (13.3)	–	1.64 (1.01–2.66)	0.045
Men[1]	344 (91.0)	34 (9.0)	–	0.86 (0.56–1.32)	0.490
Breast cancer	723 (91.1)	71 (8.9)	–	0.94 (0.71–1.26)	0.769
Women[1,2]	723 (91.1)	71 (8.9)	–	1.02 (0.72–1.44)	0.925
Prostate cancer	1063 (90.9)	106 (9.1)	–	0.96 (0.74–1.24)	0.795
Men[1,3]	1063 (90.9)	106 (9.1)	–	0.83 (0.62–1.24)	0.232

[1]Age and population adjusted.
[2]Calculations against female controls only.
[3]Calculations against male controls only.

the GG genotype. Similar genetic differences have been observed for other genes, where heterozygous and homozygous carriers may be susceptible to different diseases.[40]

In contrast to SNP309, only a few studies have explored the effect of the recently identified *MDM2* polymorphism; SNP285. The SNP285C variant resides on the SNP309G allele and creates a distinct SNP285C/309G haplotype.[16,17] This haplotype is widespread across Caucasian populations, but appear to be absent among ethnic Asians and Africans.[24] Previously, we and others have reported that the SNP285C variant may be associated with reduced risk for breast,[16,20] ovarian[16] and endometrial[19] cancer, and others reported a reduced age at cancer diagnosis related to the SNP285G allele among individuals with the Li-Fraumeni syndrome.[41] In contrast, previous reports have indicated SNP285C not to be associated with the risk of either lung[21] or prostate cancer.[19]

We found the SNP285C to be associated with reduced breast cancer risk among individuals harboring the SNP309GG genotype, but found no association among individuals harboring the SNP309TG genotype. While these findings may be surprising, both observations are in accordance with our previous findings in large independent cohorts of Norwegian as well as Dutch breast cancer patients and controls.[16] Notably, restricting the analysis to individuals from the same regions as our previous study (excluding participants from the Northern Norway), the association between SNP285C and a reduced risk for breast cancer became highly significant. Recently, we showed the SNP285C variant to be rare among individuals of Finnish and Saami ethnicity;[24] thus the altered OR may be explained by an admixture of Finnish and Saami genes in the population of Northern Norway.

Further, our findings of no association between SNP285 status and risk of prostate cancer is in accordance with our previous findings in a smaller subset of prostate cancer patients.[19] Although approximately 25% of the prostate cancer patients have been reported in a previous study, the risk assessments in this study were carried out using a different group of healthy controls.

Also, with respect to lung cancer our overall data are in line with a smaller study assessing the impact of SNP285 and SNP309 in American cancer patients.[21] Regarding colon cancer, to the best of our knowledge no previous study has addressed OR for this malignancy related to SNP285C status.

In silico predictions have shown that SNP285 is located within a half-ERE sequence overlapping with a Sp1 binding site in the *MDM2* promoter,[19] and *in vitro* experiments has shown that Sp1 and ESR1 cooperatively binds to such half-sites.[42] Based on this finding, we hypothesized the cancer risk reducing effect of SNP285C to be gender specific. Stratifying our datasets for colon and lung cancer into subgroups of females and males revealed no gender-dependent effect of SNP285C for colon cancer and a slight *increase* in the risk of lung cancer among females with SNP285C and the SNP309TG genotype. Although it has been proposed that female sex hormones are associated with lung cancer risk,[43–45] our finding may have occurred by chance, and needs to be confirmed in independent studies.

Taken together, our results obtained from a large Norwegian cohort indicate little effect of SNP309 genotypes on the OR for the four most frequent cancer forms in the Norwegian population in respect to any gender. Importantly; excluding individuals carrying the SNP285C/309G haplotype from the analysis had little impact on outcome; thus, our data indicate the presence of the SNP285C/309G haplotype among Caucasians, may not alone explain the potential discrepancy with respect to cancer risk between Caucasians and Asians as indicated in the literature.

We found no effect of SNP285C status on OR for either cancer of the lung or colon in either gender. Further, we confirmed our previous finding that SNP285C/G status may not influence the risk of prostate cancer.[19] However, we did confirm our previous finding of a reduced breast cancer risk related to the C-allele among women carrying the SNP309GG genotype.[16] Importantly, the data presented here indicate that the effect of SNP285C is likely to be tissue specific rather than gender specific. Therefore further studies evaluating the effect of SNP285C on additional cancer types are warranted.

Acknowledgements

The authors thank Beryl Leirvaag for technical assistance. Most of this work was performed in the Mohn Cancer Research Laboratory.

References

1. Haupt Y, Maya R, Kazaz A, Oren M. Mdm2 promotes the rapid degradation of p53. *Nature* 1997; 387:296–9.
2. Honda R, Tanaka H, Yasuda H. Oncoprotein MDM2 is a ubiquitin ligase E3 for tumor suppressor p53. *FEBS Lett* 1997;420:25–7.
3. Momand J, Zambetti GP, Olson DC, George D, Levine AJ. The mdm-2 oncogene product forms a complex with the p53 protein and inhibits p53-mediated transactivation. *Cell* 1992;69:1237–45.
4. Oliner JD, Pietenpol JA, Thiagalingam S, Gyuris J, Kinzler KW, Vogelstein B. Oncoprotein MDM2 conceals the activation domain of tumour suppressor p53. *Nature* 1993;362:857–60.
5. Gajjar M, Candeias MM, Malbert-Colas L, Mazars A, Fujita J, Olivares-Illana V, Fahraeus R. The p53 mRNA-Mdm2 interaction controls Mdm2 nuclear trafficking and is required for p53 activation following DNA damage. *Cancer Cell* 2012;21:25–35.
6. Zauberman A, Flusberg D, Haupt Y, Barak Y, Oren M. A functional p53-responsive intronic promoter is contained within the human mdm2 gene. *Nucleic Acids Res* 1995;23:2584–92.
7. Momand J, Jung D, Wilczynski S, Niland J. The MDM2 gene amplification database. *Nucleic Acids Res* 1998;26:3453–9.
8. Oliner JD, Kinzler KW, Meltzer PS, George DL, Vogelstein B. Amplification of a gene encoding a p53-associated protein in human sarcomas. *Nature* 1992;358:80–3.
9. Bartel F, Meye A, Wurl P, Kappler M, Bache M, Lautenschlager C, Grunbaum U, Schmidt H, Taubert H. Amplification of the MDM2 gene, but not expression of splice variants of MDM2 MRNA, is associated with prognosis in soft tissue sarcoma. *Int J Cancer* 2001;95:168–75.
10. Bond GL, Hu W, Bond EE, Robins H, Lutzker SG, Arva NC, Bargonetti J, Bartel F, Taubert H, Wuerl P, Onel K, Yip L, et al. A single nucleotide polymorphism in the MDM2 promoter attenuates the p53 tumor suppressor pathway and accelerates tumor formation in humans. *Cell* 2004;119: 591–602.
11. Bond GL, Hirshfield KM, Kirchhoff T, Alexe G, Bond EE, Robins H, Bartel F, Taubert H, Wuerl P, Hait W, Toppmeyer D, Offit K, et al. MDM2 SNP309 accelerates tumor formation in a gender-specific and hormone-dependent manner. *Cancer Res* 2006;66:5104–10.
12. Bond GL, Menin C, Bertorelle R, Alhopuro P, Aaltonen LA, Levine AJ. MDM2 SNP309 accelerates colorectal tumour formation in women. *J Med Genet* 2006;43:950–2.
13. Economopoulos KP, Sergentanis TN. Differential effects of MDM2 SNP309 polymorphism on breast cancer risk along with race: a meta-analysis. *Breast Cancer Res Treatment* 2010;120: 211–6.
14. Hu Z, Jin G, Wang L, Chen F, Wang X, Shen H. MDM2 promoter polymorphism SNP309 contributes to tumor susceptibility: evidence from 21 case-control studies. *Cancer Epidemiol Biomarkers Prev* 2007;16:2717–23.
15. Wilkening S, Bermejo JL, Hemminki K. MDM2 SNP309 and cancer risk: a combined analysis. *Carcinogenesis* 2007;28:2262–7.
16. Knappskog S, Bjornslett M, Myklebust LM, et al. The MDM2 promoter SNP285C/309G haplotype diminishes Sp1 transcription factor binding and reduces risk for breast and ovarian cancer in Caucasians. *Cancer Cell* 2011;19:273–82.

17. Paulin F, O'Neill M, McGregor G, et al. MDM2 SNP309 is associated with high grade node positive breast tumours and is in linkage disequilibrium with a novel MDM2 intron 1 polymorphism. *BMC Cancer* 2008;8:281.
18. Wu DW, Lee MC, Wang J, et al. DDX3 loss by p53 inactivation promotes tumor malignancy via the MDM2/Slug/E-cadherin pathway and poor patient outcome in non-small-cell lung cancer. *Oncogene* 2014;33:1515–26.
19. Knappskog S, Trovik J, Marcickiewicz J, et al. SNP285C modulates oestrogen receptor/Sp1 binding to the MDM2 promoter and reduces the risk of endometrial but not prostatic cancer. *Eur J Cancer* 2012;48:1988–96.
20. Piotrowski P, Lianeri M, Rubis B, et al. Murine double minute clone 2,309T/G and 285G/C promoter single nucleotide polymorphism as a risk factor for breast cancer: a Polish experience. *Int J Biol Markers* 2012;27:e105–10.
21. Ryan BM, Calhoun KM, Pine SR, et al. MDM2 SNP285 does not antagonize the effect of SNP309 in lung cancer. *Int J Cancer* 2012;131:2710–6.
22. Bjornslett M, Knappskog S, Lonning PE, Dorum A. Effect of the MDM2 promoter polymorphisms SNP309T>G and SNP285G>C on the risk of ovarian cancer in BRCA1 mutation carriers. *BMC Cancer* 2012;12:454.
23. Genomes Project C, Abecasis GR, Auton A, Brooks LD, et al. An integrated map of genetic variation from 1,092 human genomes. *Nature* 2012;491:56–65.
24. Knappskog S, Gansmo LB, Dibirova K, et al. Population distribution and ancestry of the cancer protective MDM2 SNP285 (rs117039649). *Oncotarget* 2014;5:8223–34.
25. Porter W, Saville B, Hoivik D, Safe S. Functional Synergy between the transcription factor Sp1 and the estrogen receptor. *Mol Endocrinol* 1997;11:1569–80.
26. Saville B, Wormke M, Wang F, et al. Ligand-, cell-, and estrogen receptor subtype (α/β)-dependent activation at GC-rich (Sp1) promoter elements. *J Biol Chem* 2000;275:5379–87.
27. Naess O, Sogaard AJ, Arnesen E, et al. Cohort profile: cohort of Norway (CONOR). *Int J Epidemiol* 2008;37:481–5.
28. Schmidt MK, Reincke S, Broeks A, et al. Do MDM2 SNP309 and TP53 R72P Interact in breast cancer susceptibility? A large pooled series from the breast cancer association consortium. *Cancer Res* 2007;67:9584–90.
29. Bond GL, Levine AJ. A single nucleotide polymorphism in the p53 pathway interacts with gender, environmental stresses and tumor genetics to influence cancer in humans. *Oncogene* 2007;26:1317–23.
30. Bai J, Dai J, Yu H, et al. Cigarette smoking, MDM2 SNP309, gene-environment interactions, and lung cancer risk: a meta-analysis. *J Toxicol Environ Health Part A* 2009;72:677–82.
31. Liu G, Jiang D, Shen S, Yu L. Murine double minute 2 promoter SNP309 polymorphism and prostate cancer risk: a meta-analysis. *Int J Urol* 2012;19:914–20.
32. Wan Y, Wu W, Yin Z, et al. MDM2 SNP309, gene-gene interaction, and tumor susceptibility: an updated meta-analysis. *BMC Cancer* 2011;11:208.
33. Zhao E, Cui D, Yuan L, Lu W. MDM2 SNP309 polymorphism and breast cancer risk: a meta-analysis. *Mol Biol Rep* 2012;39:3471–7.
34. Gui XH, Qiu LX, Zhang HF, et al. MDM2 309 T/G polymorphism is associated with lung cancer risk among Asians. *Eur J Cancer* 2009;45:2023–6.
35. Albuquerque C, Bakker ER, van Veelen W, Smits R. Colorectal cancers choosing sides. *Biochim Biophys Acta* 2011;1816:219–31.
36. Fu Q, Zhang G, Chen H, et al. Current evidence on the relationship between SNP309 polymorphism in the MDM2 gene and colorectal cancer risk. *Tumour Biol* 2013;34:3721–3729.
37. Fang F, Yu XJ, Yu L, Yao L. MDM2 309 T/G polymorphism is associated with colorectal cancer risk especially in Asians: a meta-analysis. *Med Oncol* 2011;28:981–5.
38. Lind H, Zienolddiny S, Ekstrom PO, Skaug V, Haugen A. Association of a functional polymorphism in the promoter of the MDM2 gene with risk of nonsmall cell lung cancer. *Int J Cancer* 2006;119:718–21.
39. Li G, Zhai X, Zhang Z, et al. MDM2 gene promoter polymorphisms and risk of lung cancer: a case-control analysis. *Carcinogenesis* 2006;27:2028–33.
40. Smirnov DA, Cheung VG. ATM gene mutations result in both recessive and dominant expression phenotypes of genes and microRNAs. *Am J Hum Genet* 2008;83:243–53.
41. Renaux-Petel M, Sesboue R, Baert-Desurmont S, et al. The MDM2 285G-309G haplotype is associated with an earlier age of tumour onset in patients with Li-Fraumeni syndrome. *Fam cancer* 2014;13:127–130.
42. Neo SJ, Su X, Thomsen JS. Surface plasmon resonance study of cooperative interactions of estrogen receptor alpha and transcriptional factor Sp1 with composite DNA elements. *Anal Chem* 2009;81:3344–9.
43. Harichand-Herdt S, Ramalingam SS. Gender-associated differences in lung cancer: clinical characteristics and treatment outcomes in women. *Semin Oncol* 2009;36:572–80.
44. Townsend EA, Miller VM, Prakash YS. Sex differences and sex steroids in lung health and disease. *Endocrine Rev* 2012;33:1–47.
45. Yao Y, Gu X, Zhu J, et al. Hormone replacement therapy in females can decrease the risk of lung cancer: a meta-analysis. *PLoS One* 2013;8:e71236.