The Single Nucleotide Polymorphism IVS1+309 in *Mouse Double Minute 2* Does Not Affect Risk of Familial Breast Cancer

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Abstract

The mouse double minute 2 (MDM2) oncoprotein promotes cell survival and cell cycle progression by inhibiting the p53 tumor suppressor protein. Further, MDM2 overexpression can inhibit DNA double-strand break repair in a p53-independent manner. Recently, it was shown that a single nucleotide polymorphism (SNP) in the *MDM2* promoter was associated with an accelerated tumor formation in individuals with a *p53* mutation. The present case-control study investigated the association of this SNP (IVS1+309) with the risk and the age of onset of familial breast cancer in patients with unknown *p53* mutation status. Data from 549 women affected by familial breast cancer and 1,065 healthy controls were analyzed. The cases did not carry *BRCA1/2* mutations. Cases and controls showed a similar genotype distribution and the SNP did not seem to modify the age of onset of familial breast cancer. The data were also examined taking into account the presence of any additional cancer after breast cancer and the family history of cases; however, no association was found. These results suggest that the SNP IVS1+309 alone affects neither the risk nor the age of onset of heritable breast cancer. (Cancer Res 2006; 66(2): 646-8)

Introduction

The human homologue of mouse double minute 2 (MDM2) is a negative regulator of the p53 tumor suppressor. The encoded protein is a nuclear phosphoprotein that binds to p53 and inhibits p53-dependent transcription (1). Overexpression of this gene can result in excessive inactivation of p53, diminishing its tumor suppressor function (2). MDM2 has also been shown to promote tumor growth in a p53-independent manner (3). The level of MDM2 protein is up-regulated in ~40% of breast cancer specimens, although *MDM2* gene amplification is uncommon in breast cancers (4, 5). Recently, Bond et al. (6, 7) analyzed the single nucleotide polymorphism (SNP) IVS1+309 (rs2279744), subsequently called SNP309, with a base change from T to G. The SNP is located in the intronic promoter of *MDM2*, which is used by both the p53 and ras pathways to activate *MDM2* transcription (8, 9). The authors showed that, in cell lines, SNP309 increases the affinity of the transcription factor Sp1 to the *MDM2* promoter and enhances the expression of MDM2 RNA and protein, which can lead to attenuation of the p53 stress response. In the study of Bond et al., 66 Li-Fraumeni patients with a germ line mutation in one allele of *p53* were examined for their genotype at the position of SNP309. Their results showed SNP309 to be associated with an early onset of different cancers. In 17 breast cancers included in this study, the age of onset was on average 10 years earlier in cases carrying a G allele in SNP309. Furthermore, in Li-Fraumeni patients first diagnosed with soft tissue sarcoma, SNP309 was associated with the occurrence of subsequent tumors. Bond et al. also reported SNP309 to be significantly associated with an earlier age of onset in patients with soft tissue sarcoma who had no known germ line *p53* mutation. This led to the assumption that, at least for soft tissue sarcoma development, SNP309 plays an important role for cancer onset, independently of germ line *p53* mutations (as reviewed in ref. 7). In Li-Fraumeni families, breast cancer is the most prevalent cancer (10). Considering the profound transcriptional effects of SNP309 and its effect on the age of breast cancer onset in Li-Fraumeni patients, we wanted to examine whether it influences familial breast cancers not known to be linked to any gene mutation. Any inherited susceptibility allele would be expected to be enriched in familial cases (8, 9), prompting our choice of familial breast cancer patients for the study.

Materials and Methods

Study population. The familial breast cancer cases consisted of 549 German women lacking *BRCA1* and *BRCA2* mutations. The cases were unrelated individuals (i.e., only one member of the family was included in the study). They were classified in four classes according to family history: F_1, families with two or more breast cancer cases, including at least two cases with onset before age 50 years; F_2, families with at least one breast cancer and at least one ovarian cancer; F_3, families with at least two breast cancer cases not included in F_1 or F_2; F_4, families with a single case of breast cancer diagnosed before age 35 years. The proportion of excluded patients with *BRCA1* or *BRCA2* mutations was 37% for F_1, 53% for F_2, and <10% for $F_3 + F_4$ (11). The probability to get breast cancer by the age of 70 years was 68% to 84% for women belonging to families in F_1, 69% in F_2, 69% to 79% in F_3, and 61% in F_4 (12). The samples were collected during the years 1997 to 2004 by the Institute of Human Genetics (Heidelberg, Germany), the Department of Gynaecology and Obstetrics (Cologne, Germany), and the Department of Medical Genetics (Munich, Germany). The control samples were collected by the Institute of Transfusion Medicine and Immunology (Mannheim, Germany) and included 1,065 healthy and

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Table 1. Analysis of the effect of *MDM2* genotype on breast cancer risk and median age of tumor onset in groups of personal and family history of breast cancer

Group	Genotype	Individuals (%)	OR (95% CI)	*P*	Median age	Quantiles, 5-95%	*P*
Personal history							
Cases (549)	TT	218 (39.7)	1.00	Reference	44	28-65	Reference
	TG	243 (44.3)	1.06 (0.85-1.33)	0.61	45	29-65	0.60
	GG	88 (16.0)	1.23 (0.90-1.68)	0.20	47	29-68	0.17
Cases with any second cancer (98, from which 79 had bilateral breast cancer)	TT	110 (42.3)	1.00	Reference	37	25-43	Reference
Controls (1,065)	TG	115 (44.2)	0.95 (0.61-1.50)	0.84	37	27-44	0.84
	GG	35 (13.5)	1.24 (0.67-2.29)	0.49	37	27-43	0.97
	TT	445 (41.8)			38	20-65	
	TG	470 (44.1)			37	19-64	
	GG	150 (14.1)			33.5	20-64	
Family history							
Cases within F_1 (200)	TT	77 (38.5)	1.00	Reference	43	30-60	Reference
	TG	91 (45.5)	1.12 (0.81-1.57)	0.48	41.5	30-62	0.71
	GG	32 (16.0)	1.30 (0.83-2.05)	0.25	45	29-61	0.67
Cases within F_2 (90)	TT	37 (41.1)	1.00	Reference	44.5	30-68	Reference
	TG	38 (42.2)	1.00 (0.62-1.62)	0.98	47	30-68	0.29
	GG	15 (16.7)	1.33 (0.70-2.50)	0.38	46	35-70	0.88
Cases within F_3 (241)	TT	96 (39.8)	1.00	Reference	47	28-69	Reference
	TG	105 (43.6)	1.05 (0.77-1.44)	0.74	47	30-65	0.90
	GG	40 (16.6)	1.28 (0.84-1.96)	0.25	52	33-77	0.05

unrelated blood donors (26-68 years of age) who shared the ethnic background with the breast cancer cases. The study was approved by the Ethics Committee of the University of Heidelberg (Heidelberg, Germany).

Genotyping. The *MDM2* SNP309 (rs2279744) was genotyped in cases and controls using a custom TaqMan genotyping assay (Applied Biosystems, Foster City, CA). Sequences are for forward primer CGGGAGTTCAGGGTAAAGGT, reverse primer GCGCAGCGTTCACACTAG, vic-labeled probe CTCCCGCGCCGAAG, and fam-labeled probe: TCCCGCGCCGCAG. For TaqMan PCR, 5 ng of genomic DNA isolated from blood was used in a total volume of 5 μL. To verify the TaqMan results, 10% of the samples were sequenced in an automated ABI PRISM 3100 genetic analyzer. Sequencing primers (forward CGGGAGTTCAGGGTAAAGGT and reverse AGCAAGTCGGTGCTTACCTG) were purchased from Thermo Electron GmbH (Ulm, Germany).

Statistical analysis. To test if the genotype distribution followed Hardy-Weinberg equilibrium, we used the public software (http://ihg.gsf.de/cgi-bin/hw/hwa1.pl). Odds ratios (OR) with the corresponding 95% confidence intervals (95% CI) and *P* values were calculated using logistic regression adjusting for age. The logistic regression analysis was done using SAS Version 8.2. Genotype-specific distributions of age of tumor onset were compared by median tests.

Results

The genotype distribution in our sample set followed the Hardy-Weinberg equilibrium (P = 0.133). Table 1 shows the genotype distribution for cases and controls, which was similar to previously published frequencies (6, 13–15). The overall OR for familial breast cancer of patients with the G/G genotype of SNP309 was 1.23 (95% CI, 0.90-1.68). To investigate the association of SNP309 with different types of heritable breast cancer, we subdivided the cases into following groups: cases with any second cancer and cases with a specific family history (F_1-F_3, for explanation see Materials and Methods). No significant association was found between the risk of breast cancer and the SNP309 genotype in any of the groups. To check whether the SNP is associated with an earlier onset of familial breast cancer, we plotted the cumulative number of cases with genotypes G/G, G/T, and T/T against the onset age of breast cancer (Fig. 1). In contrast to the results from Bond et al., the genotype G/G (●) was rather associated with a later onset of breast cancer. However, the difference between the median ages of onset was not significant (P = 0.17). The differences in age of onset among genotypes did not reach statistical significance in any of the groups.

Discussion

Bond et al. (6, 7) found SNP309 to be associated with an early onset of cancer in 66 Li-Fraumeni individuals, 17 of whom had breast cancer. Individuals who carried the G allele in SNP309 developed the first tumor 9 years earlier than individuals without this allele; for breast cancer, the difference in onset age was 10 years on average. A recent study by Bougead et al. (13)

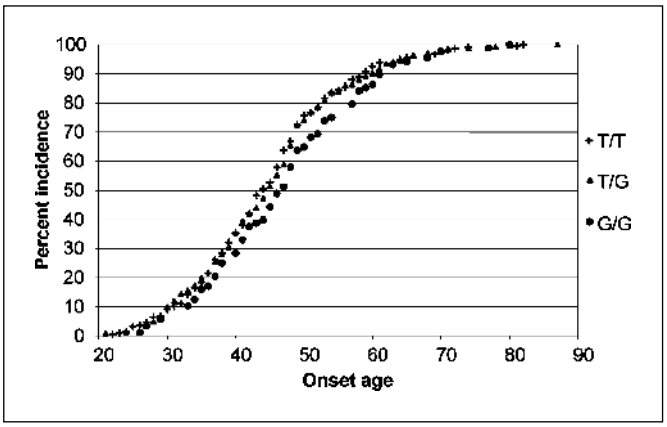

Figure 1. Cumulative number of breast cancer patients with different genotypes plotted against the age of onset.

confirmed the effect of the MDM2 SNP309 on the age of tumor onset in germ line *p53* mutation carriers. An earlier age of onset was further reported by Bond et al. for individuals with soft tissue sarcoma and no known *p53* mutation. Therefore, we hypothesized that SNP309 is also associated with the age of onset in our large collection of familial breast cancer cases, all of whom were negative for *BRCA1/2* mutations. DNA samples of patients used for this study were not scanned for *p53* mutations. However, germ line mutations in *p53* account for <1% of cases with familial breast cancer (16).

Our results suggest that SNP309 alone has little importance for the development of familial breast cancer and that additional genetic variants that weaken the p53 pathway like a *p53* mutation are needed for a significant effect. Further recent studies also showed that SNP309 alone does not have an effect on the risk or the onset of different cancers (15, 17), including breast cancer (14). Bond et al. (7) reported a significant association with SNP309 and the onset of sporadic soft tissue sarcoma in 105 individuals with no known germ line *p53* mutation. The specific association of SNP309 with soft tissue sarcoma but not with breast cancer would be indicative of a tissue-specific effect. The strength of our study was the use of DNA samples from women selected for familial breast cancer, because in these individuals the chance to find heritable cancer susceptibility alleles is much higher than in cases unselected for family history (8, 9). With our sample size, considering that ~14% of the controls carried the G/G genotype, we had an 80% power to detect an OR $\geq$1.5 (P = 0.05; ref. 18). In conclusion, our large familial case-control study suggests that SNP309 in the intronic promoter region of *MDM2* alone does not influence the risk of breast cancer.

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