

MDM2 SNP309 Accelerates Tumor Formation in a Gender-Specific and Hormone-Dependent Manner

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Abstract

The importance of the p53 stress response pathway in the suppression of tumor formation is well documented. In a previous report, a single nucleotide polymorphism (SNP309 T/G) was found in the promoter of the *MDM2* gene resulting in higher levels of MDM2 RNA and protein and, consequently, in the attenuation of the p53 pathway both *in vitro* and *in vivo*. As the SNP309 locus is found in a region of the *MDM2* promoter, which is regulated by hormonal signaling pathways, and the G-allele of SNP309 increases the affinity of a well-described cotranscriptional activator of nuclear hormone receptors (i.e., Sp1), the hypothesis that the SNP309 locus could alter the effects of hormones on tumorigenesis was tested *in vivo* in humans. Data obtained from patients with three different sporadic cancers, from four independent case studies, support this hypothesis, providing an example for the genetic basis of gender differences in cancer and showing that the genotype at a specific locus can affect how hormones, like estrogen, affect tumorigenesis in humans. (Cancer Res 2006; 66(10): 5104-10)

Introduction

On exposure to cellular stresses, the p53 protein is stabilized, increases its concentration, and becomes active as a transcription factor initiating a transcriptional program, which leads to DNA repair, cell cycle arrest, cellular senescence, or apoptosis. The p53 stress response pathway functions as a critical tumor suppressor pathway, which is underlined by the observation that the p53 gene is one of the most commonly mutated genes in human tumors (1). Furthermore, both mice and humans harboring a germ-line inactivating mutation in just one allele of the p53 gene develop tumors early in life and at very high frequencies (2–5). A previous report provided evidence that naturally occurring polymorphic genetic variant in the p53 stress response pathway influences an individual's susceptibility to cancer (6).

Both genetic and biochemical studies have shown that the *MDM2* oncogene is a key negative regulator of the p53 protein (7). Since the initial publication (6), multiple studies have shown that the G-allele of the single nucleotide polymorphism (SNP309, T/G) in the promoter of the *MDM2* gene was associated with the attenuation of the p53 pathway and an enhanced early onset of,

and increased risk for, tumorigenesis (6, 8–14). In various reports, results were presented that support the model that the G-allele of SNP309 increases the DNA binding affinity of the transcriptional activator Sp1, which results in high levels of MDM2 mRNA and protein in human cells and tissue with this allele (10, 15, 16). The heightened MDM2 levels were shown to lead to the attenuation of the p53 DNA damage response induced by various chemotherapeutic drugs (6, 15), in concordance with the well-described role of MDM2 in the negative regulation of the p53 protein. In humans, two independent reports have shown that germ-line p53 mutation carriers who possessed the G-allele of SNP309 were diagnosed with cancer, on average, 7 or 10 years earlier than those who were homozygous for the T-allele (6, 9). It was proposed that high levels of MDM2, resulting from the G-allele of SNP309 and just one wild-type p53 allele, produce a severely weakened p53 tumor suppressor pathway resulting in a higher mutation rate, poorer DNA repair processes, and reduced apoptosis leading to faster and more frequent tumor formation (6, 16).

Estrogen signaling has been shown to regulate MDM2 expression levels. Many reports have shown that MDM2 mRNA and protein levels are heightened in breast tumors which express the estrogen receptor (ER), a critical component of the estrogen signaling pathway (17–21). In fact, the expression of ER α was shown to induce transcription of MDM2 (22). The regulation of MDM2 expression by estrogen, as well as by thyroid hormone, is mediated, at least in part, through a well-characterized region of the MDM2 promoter (20, 22–24). Both the ER and the thyroid hormone receptor are known to bind to this region of the promoter and activate transcription of the MDM2 gene (23, 24). Interestingly, SNP309 is found in this region of the promoter. The SNP309 locus potentially could affect the transcriptional regulation of MDM2 by hormone receptors as the G-allele of SNP309 increases the affinity of the MDM2 promoter for Sp1, which is a well-characterized cotranscriptional activator for multiple hormone receptors, including the ER (25–27). These observations suggest the possibility that the SNP309 locus could alter the effects of hormones, like estrogen, on tumorigenesis, and therefore contribute to the gender differences observed in many different types of cancer. In this report, data are presented from four independent studies that support this hypothesis.

Materials and Methods

Statistical analysis. A randomization test is employed to determine the statistical significance of the age of onset of cancer between the different genotypes. The groups are compared pairwise and each instance of an element from the second group adds one to the distance. This total distance is the cutoff. The lists are then randomly permuted, holding fixed the number of elements of each list. The calculated *P* value is the percent of

Note: G.L. Bond and K.M. Hirshfield contributed equally to this manuscript.

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randomized groups that have a distance less than the cutoff as determined by a large Monte Carlo simulation.

Ashkenazi lymphoma and breast cancer cases. Lymphoma cases were derived from 678 cases of non-Hodgkin's lymphoma ascertained at a single center in the New York metropolitan area during the period of April 2000 to March 2005. Of these, 162 were classified as diffuse large B-cell lymphoma (DLBCL). The breast cancer cases were derived from 658 individuals with invasive ductal carcinoma (IDC). The patients were Caucasians and self-identified as of Ashkenazi Jewish ethnicity. Pathology reports, and in the case of breast tumors, ER status, of all cases were reviewed and age of diagnosis was recorded. Germ-line DNA from cases was collected and permanently stripped of identifiers before genotyping, in accord with an Institutional Review Board-approved protocol.

Controls were drawn from DNA of 976 healthy men and women, all of Ashkenazi ancestry by self-report of religion and country of origin of parents and grandparents. Age range was 18 to 65 years. Control DNA samples were available from the New York Cancer Project, an ongoing cohort study of over 17,000 volunteers of varying ethnic backgrounds who live in the New York metropolitan area (28).

Soft-tissue sarcoma cases. Samples originated from 105 sporadic soft-tissue sarcoma (STS) cases diagnosed from 1991 to 2001 at the Surgical

Clinic 1, University of Leipzig and at the Institute of Pathology of the Martin-Luther-University Halle, Germany. All patients had a R0-resection of a their primary tumor done by the same team. The patients were of Caucasian ethnicity with an age range of 14 to 84 years (average, 55 years). The STS samples consisted of 29 liposarcomas, 23 malignant fibrous histiocytomas, 16 leiomyosarcomas, 12 neurogenic sarcomas, 8 rhabdomyosarcomas, 8 synovial sarcomas, 5 fibrosarcomas, and 4 other types. One hundred four healthy blood donors of Caucasian ethnicity (non-Jewish) from Germany served as controls.

Breast cancer cases. Between April 2004 and October 2005, 258 Caucasian women previously diagnosed with infiltrating ductal carcinoma of the breast were accrued to this study at The Cancer Institute of New Jersey (New Brunswick, NJ). Pathology reports were reviewed on all cases. Venipuncture was done and genomic DNA was prepared from whole blood. Age and menopausal status at diagnosis, ER status, and degree of positivity were recorded. All information was devoid of identifiers and kept in a database. Data collection was in accord with an approval by the Institutional Review Board at the Cancer Institute of New Jersey.

Sequence analysis. The status of the SNP309 locus was determined for the p53 germ-line mutation carriers, the unaffected non-Ashkenazi Jewish individuals, the STS patients, and the second group of Caucasian IDC

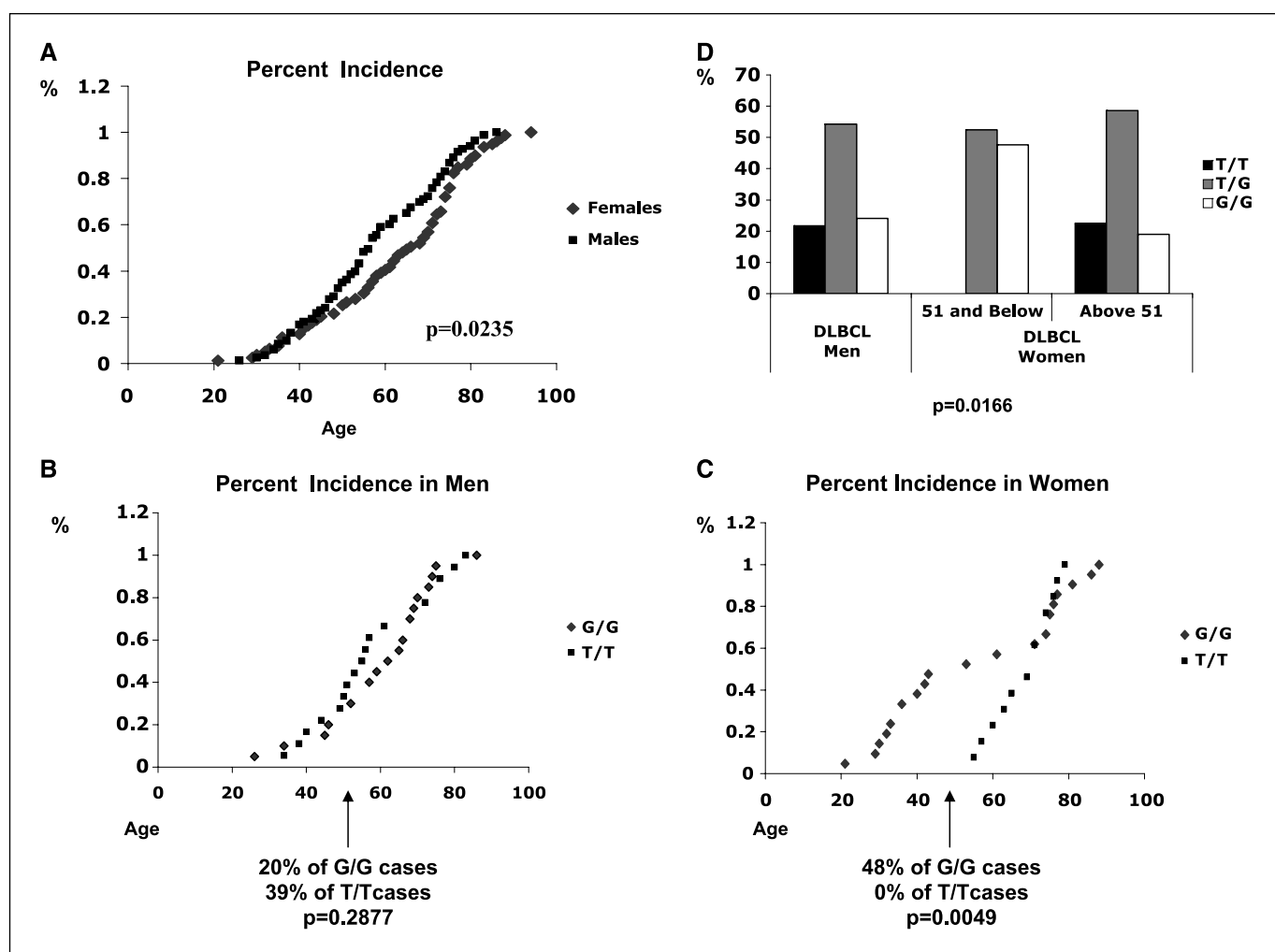


Figure 1. The G-allele of SNP309 associates with an accelerated age of onset of DLBCL in younger females but not in males. DLBCL has a well-documented gender difference in tumor incidence. *A*, the cumulative incidence of cancer for both men (black squares) and women (gray diamonds) is plotted as a function of age. The cumulative incidence of DLBCL for both the individuals T/T in genotype (black squares) and G/G in genotype (gray diamonds) is plotted as a function of age for males (*B*) and females (*C*). Female DLBCL patients diagnosed below the average age of menopause (51 years) are enriched for the G-allele of SNP309. *D*, relative ratios of the three genotypes at the SNP309 locus for the male DLBCL patients, the female DLBCL patients diagnosed at ≤ 51 years of age, and females diagnosed >51 years of age.

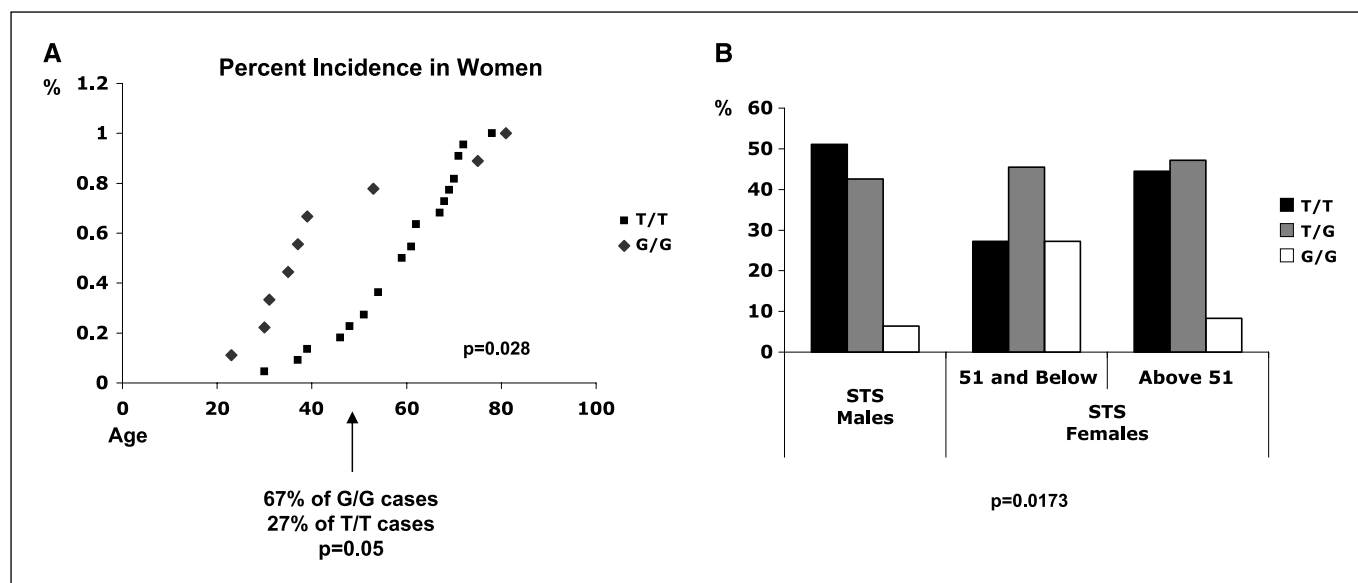


Figure 2. The G-allele of SNP309 associates with an accelerated age of onset of STS in younger females. *A*, the cumulative incidence of STS for both the women T/T in genotype (black squares) and G/G in genotype (gray diamonds) is plotted as a function of age. Female STS patients diagnosed below the average age of menopause (51 years) are enriched for the G-allele of SNP309. *B*, the relative ratios of the three genotypes at the SNP309 locus for the male STS patients, the female STS patients diagnosed at ≤ 51 years of age, and females diagnosed >51 years of age.

patients by PCR amplification and subsequent sequencing (primer 1, CGGGAGTTCAGGGTAAAGGT; primer 2, AGCAAGTCGGTGCTTACCTG).

The status of the SNP309 locus was determined for the unaffected Ashkenazi Jewish individuals, DLBCL patients, and the first group of Caucasian Ashkenazi Jewish IDC patients by using the combination of *Msp*I1 RFLP analysis and 5' allelic discrimination assay (TaqMan). For the *Msp*I1 RFLP analysis, primers 5'-CGGGAGTTCAGGGTAAAGGT-3' and 5'-AGCAAGTCGGTGCTTACCTG-3' were used. PCR was done under standard conditions using 20 ng of genomic DNA and annealing temperature of 66°C. The resulting PCR product (351 bp) was digested by *Msp*I1. *Msp*I1 cleaves final PCR product on two sites, one is constitutive that served as an internal control of enzymatic digestion and allele G of SNP309 generates specific *Msp*I1 restriction site.

The TaqMan 5' allelic discrimination PCR assay was done in 384-well plates. Each well contained 5.0 ng DNA, 2.5 μ L TaqMan Universal PCR Master Mix (Applied Biosystems, Foster City, CA), 0.0625 μ L of probe and primer solution (Assays-on-Demand, Applied Biosystems), and 2.4375 μ L distilled water. The PCR reaction was initiated at 95°C for 10 minutes, followed by 47 cycles of 92°C for 30 seconds and 60°C for 60 seconds. Following PCR, fluorescence was measured in an ABI 7900 HT Sequence Detector (Applied Biosystems) and the genotype clusters were manually scored using Sequence Detection Software 2.0 (Applied Biosystems).

Results

Diffuse large B-cell lymphoma. To test if SNP309 can contribute to the gender differences observed in cancer, a type of cancer was studied with a well-documented gender difference in tumor incidence (i.e., non-Hodgkin's lymphoma). Non-Hodgkin's lymphoma is the fifth most common cancer in both men and women but men have an increased risk for developing non-Hodgkin's lymphoma worldwide, as well as an earlier average age of tumor diagnosis (29, 30). In this study, Caucasians of Ashkenazi Jewish ethnicity with DLBCL were analyzed. It is interesting to note that Ashkenazi Jewish individuals have a higher relative frequency of the G-allele compared with Caucasians not of Ashkenazi descent. Specifically, 976 Ashkenazi Jewish individuals who had never been diagnosed with cancer, on sample ascertainment, were

found to have the following relative frequency of three different genotypes at the SNP309 locus: T/T, 27%; T/G, 49%; G/G, 24%. One hundred four Caucasians, not of Ashkenazi descent and who had never been diagnosed with cancer, on sample ascertainment, were found to have the following relative frequencies: T/T, 38%; T/G, 51%; G/G, 10%.

As previously observed (29, 30), in 162 Caucasians of Ashkenazi Jewish ethnicity diagnosed with DLBCL, the 83 male patients were diagnosed, on average, 5 years earlier than the 79 female patients (Fig. 1A). Specifically, the male patients were diagnosed, on average, at 57 years of age (range, 25-85 years) and females at 62 years of age (range, 21-93 years). The male DLBCL patients showed no large differences in the age of tumor diagnosis when separated into the different genotypes of the SNP309 locus (T/T males, 58 years of age versus G/G males, 60 years of age; Fig. 1B). In contrast, G/G women showed a 13-year earlier tumor diagnosis than T/T women [T/T women, 68 years of age (range, 55-78 years) versus G/G women, 55 years of age (range, 21-87 years); Fig. 1C].

Estrogen has been proposed to play a role in the gender-specific differences in DLBCL incidence, as women exposed to exogenous estrogens have been observed to have altered risk for DLBCL (31-33). Indeed, ER, a critical component of the estrogen-signaling pathway, is expressed in multiple B-cell lymphomas (34-36). If estrogen signaling is working to allow the G-allele of SNP309 to accelerate tumor formation in women, then differences in the age-dependent incidence of DLBCL between G/G women and T/T women should be the largest in women below the average age of menopause (51 years) when female specific hormones like estrogen are at their highest levels. As seen in the Fig. 1C, where the cumulative incidence of DLBCL for each genotype is plotted as a function of age, the greatest differences in the age-dependent incidence between the two genotypes are seen in women <51 years of age. Indeed, there are no women with a T/T genotype in these cases diagnosed with DLBCL under the age of 55 years. By contrast, over half of the G/G women had already been diagnosed with DLBCL by this age ($P = 0.0027$, two-tailed Fisher's exact test). The

G/G women make up 48% of the female DLBCL cases diagnosed by the age of 51 years and only 19% of the female cases >51 years of age and only 24% of the male DLBCL cases (Fig. 1D; $P = 0.0166$, two-tailed Fisher's exact test). Together, these data support the hypothesis that estrogen could play a role in the ability of the G-allele of SNP309 to accelerate DLBCL formation in women as the greatest differences in the age-dependent incidence between G/G women and T/T women are the greatest below the average age of menopause when gender-specific hormones, like estrogen, are at the highest levels.

Soft-tissue sarcoma. The G-allele of SNP309 in its homozygous state (G/G) has been previously shown to associate with a 12-year earlier age of onset of sporadic STS compared with T/T individuals (6). The STS patients in this study were made up of 58 Caucasian women and 47 Caucasian men. When separated by gender, it became clear that the significant earlier age of onset of STS observed in these cases is only associated with the G/G women, as only 3 of 47 male patients had the G/G genotype. Specifically, G/G women were diagnosed on average 14 years earlier than T/T women [T/T women, 59 years of age (range, 30-78 years) versus G/G women, 45 years of age (range, 23-81 years); Fig. 2A; $P = 0.028$, randomization test]. As the presence of ER in STS is well documented (37-40), the possibility that estrogen might be playing a role in the ability of the G-allele of SNP309 to accelerate STS formation in women was tested. Indeed, the largest differences in the age-dependent STS incidence between G/G women and T/T women were also seen in women below the average age of menopause (51 years) when gender-specific hormones, such as estrogen, are at their highest levels. Specifically, 67% of G/G women were diagnosed with STS by the age of 51 years, but only 27% of T/T women ($P = 0.05$, one-tailed Fisher's exact test). In fact, the overall frequency of the G/G genotype is 27% in female STS cases diagnosed by the age of 51 years and only 8% in female cases diagnosed >51 years of age and 6% in male STS cases (Fig. 2B; $P = 0.0173$, one-tailed Fisher's exact test). Together these data further support the hypothesis that gender-specific hormones, like estrogen, could be playing a role in the ability of the G-allele of SNP309 to accelerate both sporadic DLBCL formation and sporadic STS tumor formation.

Invasive ductal breast carcinoma. To test if the G-allele of SNP309 can indeed accelerate tumorigenesis only in the presence of an active estrogen-signaling pathway *in vivo*, women with the same tumor type were studied. The tumor type studied was sporadic IDC of the breast, which accounts for the vast majority of all breast cancers (41). Some women developed IDC with an intact estrogen pathway whereas others did not. DNA was isolated from lymphocytes from women with IDC of the breast and the status of SNP309 was determined. All patients were Caucasians of self-identified Ashkenazi Jewish ethnicity. Individuals with the most common breast cancer susceptibility alleles of the *BRCA1* and *BRCA2* genes were excluded from this study. In each case, pathology reports, including analysis of ER (when available), and age at diagnosis were recorded. At diagnosis, the levels of the ER, a critical component of the estrogen-signaling pathway, are measured in the tumor to determine the most appropriate course of therapy. ER is detectable in 60% to 70% of all breast cancers and its expression level correlates with the tumor dependence on estrogen for growth (42, 43).

Of the 658 IDC patients, 136 patients were noted to have no significant levels of ER expression (<10% of tumor cells stained positive for ER) whereas 472 were noted to have significant levels of ER staining ($\geq 10\%$ of tumors cells stained positive for ER) and the expression level of ER was not known for 50 patients. Of the 472

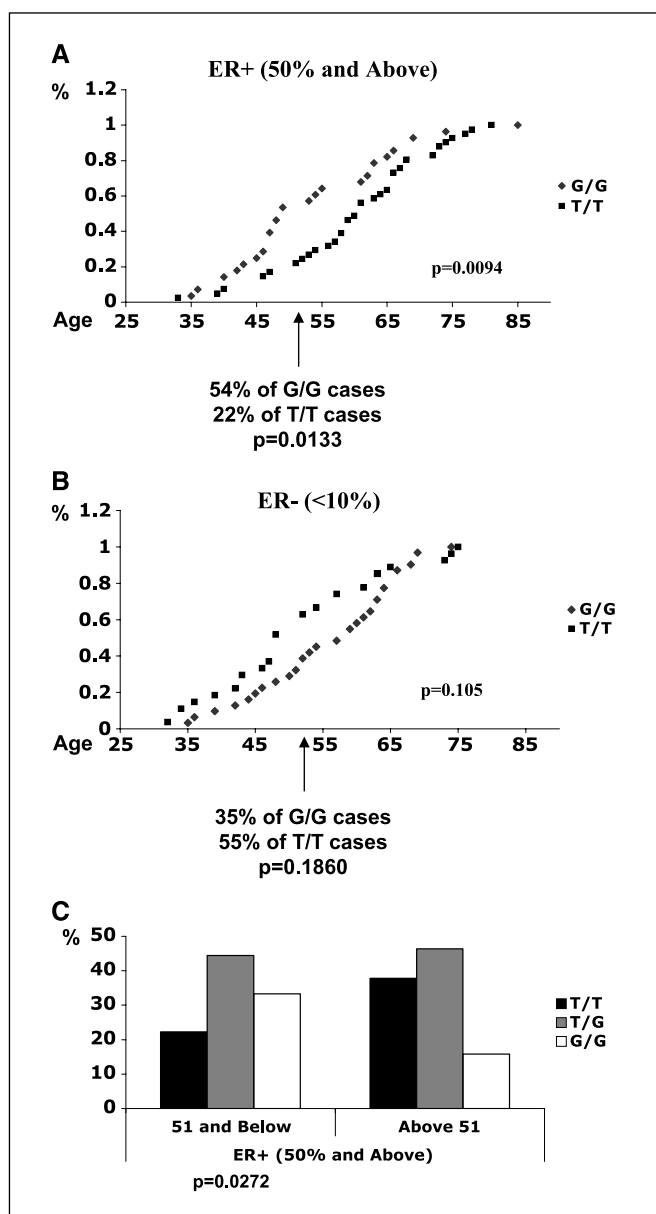


Figure 3. The G-allele of SNP309 associates with an accelerated age of onset in high ER-positive, but not in ER-negative, IDC of the breast in Caucasians (Ashkenazi Jewish). The cumulative incidence of IDC for both the individuals T/T in genotype (black squares) and G/G in genotype (gray diamonds) is plotted as a function of age for high ER-positive tumors (>50% of the tumor cells stained positive for ER; A) and ER-negative tumors (<10%; B). Female patients with high ER-positive IDC (>50% of the tumor cells stained positive for ER) diagnosed below the average age of menopause (51 years) are enriched for the G-allele of SNP309. C, relative ratios of the three genotypes at the SNP309 locus for the female high ER-positive IDC patients diagnosed at ≤ 51 years of age and females diagnosed >51 years of age.

patients noted to have significant levels of ER staining, 127 patients were known to have high levels of ER expression ($\geq 50\%$ of the tumor cells stained positive for ER).

If the G-allele of SNP309 can accelerate tumorigenesis only in the presence of an active estrogen-signaling pathway, then an earlier age of tumor onset for G/G women versus T/T women should be greatest in the formation of tumors that express high levels of ER, as the percent of tumor cells which stain positive for ER has been shown to correlate with the tumor dependence on estrogen for growth (42, 43). Indeed, in those 100 women whose tumors

expressed high levels of ER ($\geq 50\%$), G/G women showed a 7-year average earlier age of onset of IDC compared with T/T women and an 11-year median earlier onset [T/T women, 60 years of age (range, 33-81 years) versus G/G women, 53 years of age (range, 35-84 years); $P = 0.0094$, randomization test; Fig. 3A]. No significant differences were observed in those women whose tumors expressed low levels of ER [$<10\%$ (Fig. 3B) or $<50\%$ (data not shown)]. Specifically, the ER-negative T/T patients were diagnosed on average at 51 years of age and G/G patients at 56 years of age. Interestingly, the acceleration of high ER-positive ($\geq 50\%$) IDC formation in G/G women compared with T/T women was also greatest below the average age of menopause (51 years) when estrogen levels are at their highest. Fifty-four percent of the patients with the G/G genotype had been diagnosed with high ER-positive ($\geq 50\%$) IDC by the age of 51 years, in contrast to only 22% of the T/T patients (Fig. 3A; $P = 0.0133$, one-tailed Fisher's exact test). In fact, women with the G/G genotype make up 33% of high ER-positive ($\geq 50\%$) IDC cases diagnosed by the age of 51 years, as opposed to 16% of high ER-positive ($\geq 50\%$) IDC cases diagnosed

>51 years of age (Fig. 3C; $P = 0.0272$, two-tailed Fisher's exact test). These data further support the hypothesis that an active estrogen-signaling pathway allows for the G-allele of SNP309 to accelerate tumor formation in women.

To confirm these observations with an independent study, the hypothesis was tested again in a second group of Caucasian IDC patients not selected for Ashkenazi Jewish ethnicity. In the 258 IDC patients in this study, 71 patients were noted to have no significant levels of ER expression ($<10\%$ of tumor cells stained positive for ER) whereas 184 were noted to have significant levels of ER staining ($\geq 10\%$ of tumor cells stained positive for ER) and the expression level of ER was not known for 3 patients. Of the 184 patients noted to have significant levels of ER staining, 117 patients were known to have high levels of ER expression ($\geq 50\%$ of the tumor cells stained positive for ER).

As previously observed, in the 117 women in this second group whose tumors expressed high levels of ER ($>50\%$), G/G women again showed a 7-year earlier onset of IDC compared with T/T women [T/T women, 54 years of age (range, 29-76 years) versus

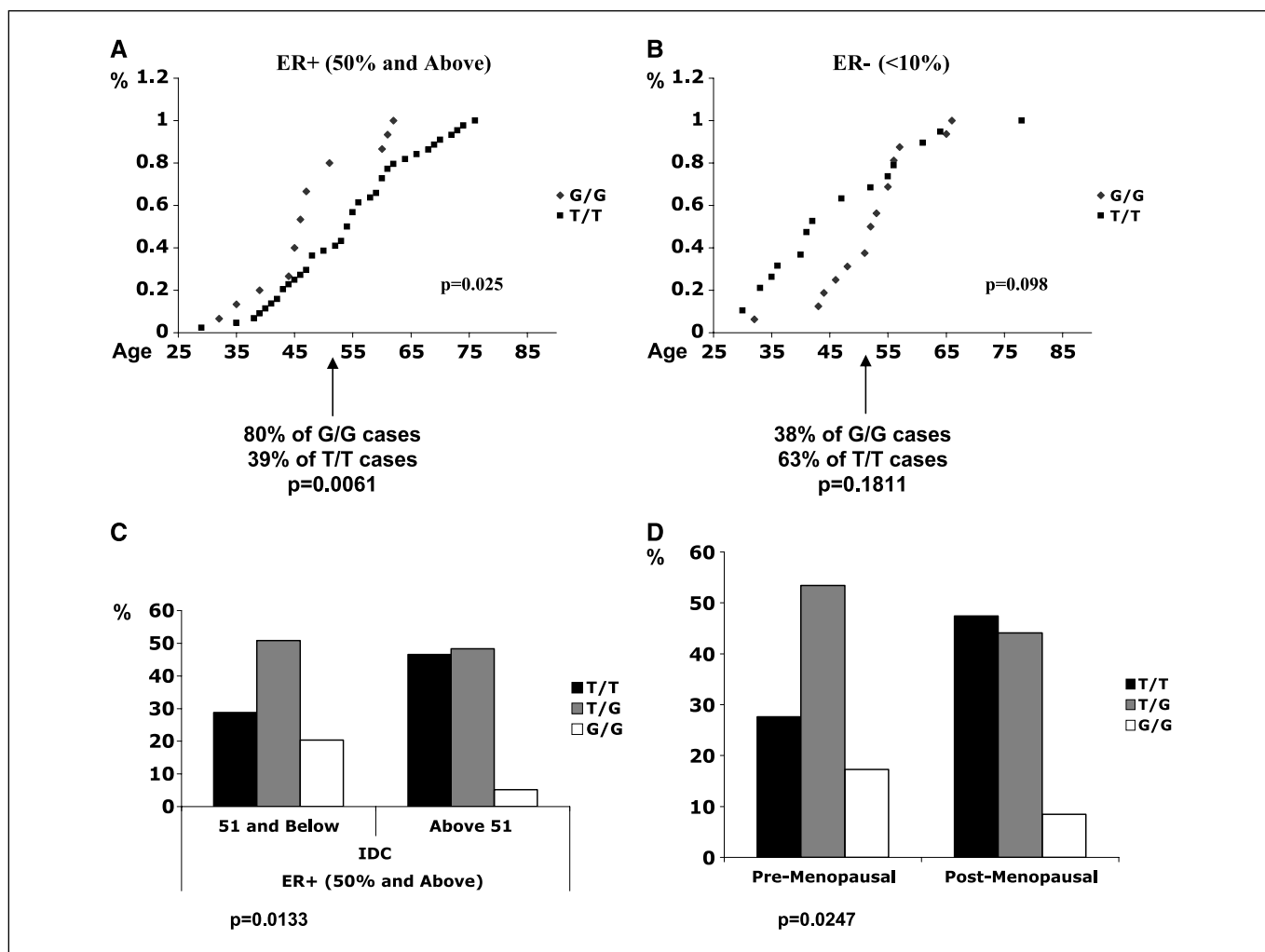


Figure 4. The G-allele of SNP309 associates with an accelerated age of onset in high ER-positive but not ER-negative IDC of the breast in a second independent group of Caucasians. The cumulative incidence of IDC for both the individuals T/T in genotype (black squares) and G/G in genotype (gray diamonds) is plotted as a function of age for high ER-positive tumors ($>50\%$ of the tumor cells stained positive for ER; A) and ER-negative tumors ($<10\%$; B). Female patients with high ER-positive IDC ($>50\%$ of the tumor cells stained positive for ER) diagnosed below the average age of menopause (51 years; C) or premenopausal (D) are enriched for the G-allele of SNP309. C and D, relative ratios of the three genotypes at the SNP309 locus for the female high ER-positive IDC patients diagnosed at ≤ 51 years of age or premenopausal and females diagnosed >51 years of age or postmenopausal.

G/G women, 47 years of age (range, 32-62 years); $P = 0.025$, randomization test; Fig. 4A]. Again, no significant differences were observed in the 71 women whose tumors expressed lower levels of ER [$<10\%$ (Fig. 4B) or $<50\%$ (data not shown)]. The acceleration of high ER-positive ($\geq 50\%$) IDC formation in G/G women compared with T/T women was also greatest below the average age of menopause (51 years) when estrogen levels are at their highest. Eighty percent of the G/G patients had been diagnosed with ER-positive IDC by the age of 51 years, but only 39% of the T/T patients ($P = 0.0061$, one-sided Fisher's exact test). In fact, G/G women make up 20% of high ER-positive ($\geq 50\%$) IDC cases diagnosed by the age of 51 years, as opposed to 5% of high ER-positive ($\geq 50\%$) IDC cases diagnosed >51 years of age (Fig. 4C; $P = 0.0133$, one-sided Fisher's exact test). In this study, the menopause status of the patients at diagnosis was available and, as predicted, individuals with the G-allele of SNP309 were enriched in the premenopausal high ER-positive ($\geq 50\%$) IDC cases compared with the postmenopausal cases. Specifically, individuals with the G-allele of SNP309 made up 72% of the premenopausal cases and only 53% of the postmenopausal cases (Fig. 4D; $P = 0.0247$, one-sided Fisher's exact test).

Discussion

In summary, the results of four independent studies of three different sporadic cancers (DLBCL, STS, and IDC) support the model that an active estrogen-signaling pathway, either directly or indirectly, allows for the G-allele of SNP309 to accelerate tumor formation in women. Specifically, in DLBCL patients (Fig. 1), the G-allele of SNP309 only associated with an earlier age of diagnosis in female patients and not in male patients, similar to what was previously observed in a study of male and female colorectal cancer patients (8). That this gender-specific difference could be due to gender-specific hormones was supported by the observations that both in female DLBCL patients (Fig. 1) and in female STS patients (Fig. 2), the differences in age-specific cancer incidence between G/G and T/T women were the largest below the average age of menopause (51 years) when gender-specific hormones, like estrogen, are at their highest levels. That the estrogen-signaling pathway could be either directly or indirectly involved was supported by the observations that the G-allele of SNP309 only

associated with an earlier age of onset in high ER-positive ($\geq 50\%$), but not in ER-negative, IDC formation, which was seen in two independent case studies (Figs. 3 and 4). Further support came from the observation that the differences in age-specific cancer incidence between G/G and T/T women with high ER-positive ($\geq 50\%$) IDC were the largest below the average age of menopause (51 years) when estrogen levels are at their highest (Figs. 3A and 4A). This model, in which an active estrogen-signaling pathway allows for the G-allele of SNP309 to accelerate tumor formation in women, may help explain the genetic basis for the frequently observed gender differences in cancer and shows that the genotype at a specific locus can affect how hormones, like estrogen, will affect tumorigenesis in humans.

The estrogen-signaling pathway can be regulated in humans for a variety of reasons (e.g., contraception, relief of menopausal symptoms, as well as cancer prevention and treatment). Estrogen signaling manipulation has been shown to alter both cancer incidence and progression (42-44). This study suggests that women with a G/G genotype for SNP309 could be affected differently by estrogen signaling manipulation than women with a T/T genotype. Specifically, in the case studies of DLBCL, STS, and high ER-positive ($\geq 50\%$) IDC of the breast, a total of 147 female cancer patients diagnosed below the average age of menopause, when estrogen levels are at their highest, were greatly and significantly enriched for women with a G/G genotype (29.3%) when compared with the 234 female patients diagnosed after the average age of menopause (12.8% G/G; $P < 0.0001$, two-sided Fisher's exact test) when estrogen levels in women measurably decrease (Figs. 1D, 2B, 3C, and 4C; Table 1). These observations suggest that increasing estrogen levels in postmenopausal women with a G/G genotype, to alleviate menopausal symptoms, could significantly increase their risk to develop these cancers. Indeed, studies have shown the association of exogenous estrogen use by menopausal women leads to an increased risk of developing ER-positive IDC and other cancers (44-46). In contrast, these observations imply that women with a G/G genotype and these cancers (DLBCL, STS, and high ER-positive IDC) would benefit from decreasing estrogen levels and this could significantly retard the progression of their disease. Indeed, many studies have shown that reducing estrogen signaling in patients with high ER-positive IDC significantly retards tumor

Table 1. Distribution of MDM2 SNP309 in patients from four independent case studies of three different tumor types

	DLBCL, n (%)		STS, n (%)		IDC, n (%)		IDC, n (%)		Total, n (%)			
	Males	Females	Males	Females	ER+ ($\geq 50\%$)	ER+ ($\geq 50\%$)	ER+ ($\geq 50\%$)	Females	ER+ ($\geq 50\%$)	Females		
		≤ 51 y		>51 y		≤ 51 y		>51 y		≤ 51 y	>51 y	≤ 51 y
T/T	18 (22%)	0 (0%)	13 (22%)	24 (51%)	6 (27%)	16 (44%)	10 (22%)	31 (38%)	17 (29%)	27 (47%)	33 (22.4%)	87 (37.2%)
T/G	45 (54%)	11 (52%)	34 (59%)	20 (43%)	10 (45%)	17 (47%)	20 (44%)	38 (46%)	30 (51%)	28 (48%)	71 (48.3%)	117 (50.0%)
G/G	20 (24%)	10 (48%)	11 (19%)	3 (6%)	6 (27%)	3 (8%)	15 (33%)	13 (16%)	12 (20%)	3 (5%)	43 (29.3%)	30 (12.8%)
Total	83	21	58	47	22	36	45	82	59	58	147	234
P value for enrichment of G/G	$P = 0.0166$		$P = 0.0173$		$P = 0.0272$		$P = 0.0133$		$P < 0.0001$			
Fisher's exact test	two-tailed		one-tailed		two-tailed		one-tailed		two-tailed			
Race	Caucasian		Caucasian		Caucasian		Caucasian		Caucasian		Caucasian	
Ethnicity	Ashkenazi Jewish						Ashkenazi Jewish					

growth and results in longer overall survival rates (43, 47). It will be interesting to determine if reduction of estrogen signaling will also prove to be an effective treatment for G/G women with DLBCL and STS, as the results presented here predict.

Acknowledgments

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