

MDM2 SNP309 G allele increases risk but the T allele is associated with earlier onset age of sporadic breast cancers in the Chinese population

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Sporadic breast cancer in women <40 years is uncommon in Caucasians, in contrast to a much earlier onset in Chinese Asians. However, the molecular determinants for this earlier onset are unclear. It has been reported that SNP309 in the promoter of *MDM2*, the negative regulator of p53, affects the onset age of cancers in females. Essentially, the G allele, rather than the T allele, has been suggested to accelerate the age of cancer onset. Hence, we examined if *MDM2* and p53 polymorphisms would be determinants of the early onset phenomenon in Chinese women. Our results indicate that the *MDM2* SNP309 G allele is more prevalent in the Chinese population compared with reported frequencies in Caucasians, and increases breast cancer risk of both sporadic cases and those with family history. However, it was the T/T genotype that was associated with earlier onset age of sporadic breast cancers in contrast to the G allele that was associated with the familial cases. Though p53 codon 72 single-nucleotide polymorphism (SNP) did not affect general cancer risk or age of onset, arginine homozygosity, in contrast to proline homozygosity, was found to decrease breast cancer risk in the later onset sporadic cases. Both SNP309 and codon 72 polymorphisms did not affect the stage of cancer. Together, the data suggest that though the *MDM2* SNP309 G allele is a risk factor for breast cancer, it does not accelerate, but delays the onset of the sporadic disease in Chinese women, highlighting that differences in ethnicity and family history may influence the role of *MDM2* SNP309 in cancer susceptibility.

Introduction

Breast cancer is one of the major cancers affecting morbidity and mortality of females worldwide (1). It is often associated with postmenopausal stage, surfacing generally around or after 50 years of age. The median age in the Caucasian population is ~61 years, where >75% of the cases occur in women >50 years of age (2,3). Breast cancer incidence <40 years of age is uncommon in most western Caucasian populations, besides those that are associated with hereditary familial cases (2,4). However, though breast cancers still occur in postmenopausal women, with >50% of the cases occurring in women >50 years of age (5), there is an emerging trend in the Asian Chinese populations where there is an increase in the number of women succumbing to the disease at a much earlier age (6,7). This earlier onset of the disease, which is non-familial, is seen in women starting from 20 years of age, leading to a large number of cases

Abbreviations: Arg, arginine; ER, estrogen receptor; OR, odds ratio; PCR, polymerase chain reaction; Pro, proline; SNP, single-nucleotide polymorphism.

occurring in women under the age of 40 years (5). Thus, there appears to be a shift in the onset age, leading to the suggestion that the distribution in the Asian Chinese population follows a bimodal pattern (Figure 1) (6). Hitherto, the causes or the genetic basis for the earlier onset of breast cancers in Asians are not understood.

p53 is a critical tumor suppressor gene that is commonly mutated in many human cancers (8). In breast cancers, it is mutated in around 30–35% of the cases and mutations often lead to poorer prognosis (9,10). Moreover, Li–Fraumeni patients with germ line p53 mutations often develop breast cancers (11,12), highlighting an important role for this protein in regulating cancer predisposition. Besides mutations, a common single-nucleotide polymorphism (SNP) in codon 72 of p53, resulting in either an arginine (Arg) or proline (Pro) residue, has been demonstrated to affect p53 function. The Arg polymorphic variant has been shown to induce apoptosis better than the Pro form, and conversely, the Pro form was shown to have a higher capacity for DNA repair and induction of cell cycle arrest than the Arg form (13–15). Consistent with its functional relevance, this polymorphism has been implicated in cancer predisposition, though literature is divided over the role of either variant in affecting cancer risk. An initial report suggested a positive correlation between the Arg allele and susceptibility to cancer (16). However, subsequent reports have both supported the initial findings or found no such correlation (17–25), which could perhaps be due to ethnic variations of the frequency of these alleles in different healthy populations (26). Recently, we have demonstrated that although there were no significant differences of the genotypic frequency between healthy controls and breast cancer cases among the Asian Chinese population, the expression of the Arg allele was elevated in Arg/Pro heterozygote breast cancers, suggesting a positive role for this allele in cancer predisposition (27).

Similar to SNPs affecting the function of p53, polymorphic variants with functional relevance have been identified in many upstream and downstream genes of the p53 pathway. For example, polymorphisms in *AKT* and *MDM2* have been recently found to affect cellular response (28,29). Of note, the SNP in the promoter of *MDM2*, the negative regulator of p53, has been intensely studied (28,30,31). This SNP309, resulting in either a T or G allele, was shown to affect SP-1 binding, thereby modulating *MDM2* transcription, and hence, its levels (28). The effect on *MDM2* levels have a snow-balling effect on p53 amounts in the cell, and consistently, the G allele which leads to higher *MDM2* transcription was shown to attenuate the p53 response (28). Because the SP-1-binding site is found in the estrogen receptor (ER)-binding motif on the *MDM2* promoter, it was suggested that the G allele would be more sensitive to estrogen response compared with the T allele. Consistently, Bond *et al.* (30) searched for and found a correlation between the G allele and earlier onset of three different cancers in younger females, who would generally have higher estrogen levels than their older postmenopausal counterparts.

One could therefore envisage that the earlier onset of non-familial breast cancers in the Asian population could be associated with the *MDM2* and/or p53 polymorphisms that would weaken the p53 pathway. In an attempt to find a genetic basis to this phenomenon, we have investigated the status of the common p53 and *MDM2* polymorphic alleles in non-familial breast cancer cases occurring from 20 to 86 years of age (both earlier and later onset cases), comparing it with healthy controls from the Asian Chinese population. Our data suggest that whereas the p53 codon 72 SNP does not affect the age of onset, the *MDM2* SNP309 G allele, which is highly prevalent in the healthy Chinese controls compared with the reported frequencies in Caucasians, is associated with higher cancer risk, regardless of the age of onset of the disease. However, contrary to earlier reports, the G allele was not associated with the earlier onset of sporadic cancers. In contrast, it was the T allele of *MDM2* SNP309 that was associated with

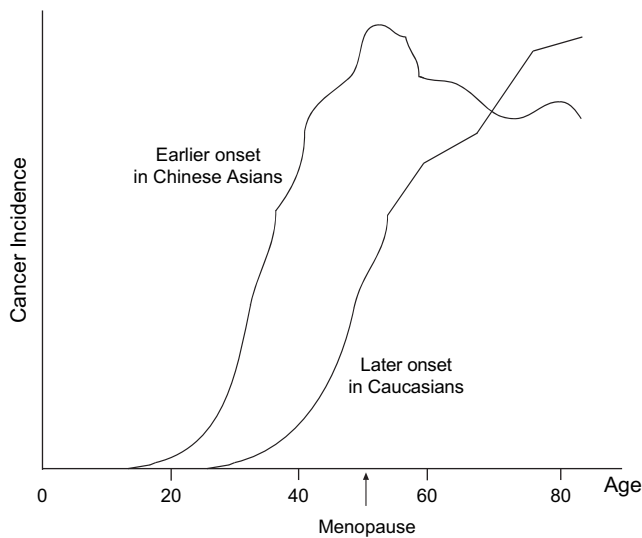


Fig. 1. Schematic representation of breast cancer incidence in different populations. The schematic indicates the earlier occurrence of breast cancers in the Chinese Asians, based on the epidemiological data (adapted from ref. 5). It represents patients who succumb to earlier onset, non-familial cancers, usually occurring between the ages of 20 and 40 and those contracting cancer later at about the postmenopausal stage. In contrast, non-familial breast cancers often occur later in life in the western Caucasian populations. This disease is rare and uncommon in patients <40 years of age in the western population. Not to scale.

earlier onset age of the disease. These data therefore highlight that ethnic differences may influence the effect of *MDM2* SNP309 on cancer predisposition and raises the possibility that other mechanisms may be in operation that leads to a correlation between the T allele of *MDM2* SNP309 with earlier onset of breast cancer in Chinese Asians.

Materials and methods

Samples

Genomic DNA was prepared from peripheral blood as described by standard procedures using the Qiagen kit (27). We collected healthy samples from a total of 44 Singaporean Chinese and 84 healthy Shanghai Chinese females. The Singaporean samples were part of an earlier study analyzing the risk of breast cancer (27,32). Subjects were healthy volunteers and those with history of gastrointestinal disorders and liver dysfunction were excluded from the study. Breast cancer samples were only collected from the Shanghai population, which included samples of various ages from 449 female breast cancer patients. All materials were collected with the approval of the National Cancer Centre and Fudan University ethics committees and used for genotyping. All the cancer cases came from independent families, between the years 2000 and 2005, and all of them received the standard treatment. Normal controls were over matched women who had no personal or family history of any cancer until 60 years of age. All samples (both healthy and cancer) were collected from Shanghai and the neighboring Shandong province, which is on the north of Shanghai, in the same time period. There were no significant differences in demographics between cases and sporadic healthy controls. The cases and controls were not matched designedly, but are only consecutive patients who consented to be involved in our study. The family history was defined as at least one first- or second-degree relative with breast and/or ovarian cancer, regardless of age. The family histories were retrieved from the medical records and standard questionnaires, ascertained by the families and/or the patients personally. Data on stage and age of onset of disease, family history, etc. were obtained from the hospital records with ethics approval.

Genotyping and sequencing

Genomic DNA from peripheral blood samples was analyzed for the genetic variation in codon 72 in exon 4 of the *p53* gene, by polymerase chain reaction (PCR) analysis followed by BstUI digestion, using primers described (27). The *p53* arg allele has a unique BstUI site that is absent in the *pro* allele, resulting in bands of different sizes (27).

MDM2 promoter SNP309 was determined by sequencing reactions. Briefly, a part of the *MDM2* promoter (320 bases) was amplified using the following PCR primers—external primers: sense, 5'-CGGGAGTTCAGGGTAAAGGT-3' and antisense, 5'-AGCAAGTCGGTGCTTACCTG-3'. PCR amplification was performed in 25 μ l reactions using 5 μ l of Q buffer (Qiagen), 2.5 μ l of 10 \times PCR buffer (Qiagen, Hilden, Germany), 0.4 μ M of each primer, 0.4 μ M of deoxy-nucleotide triphosphates, 1.5 U of *Taq* polymerase (Qiagen) and genomic DNA, using the following PCR conditions: 94°C (80 s), 30 cycles of 94°C (25 s), 51°C (60 s), 72°C (60 s) and 72°C (8 min). PCR product was purified via a purification kit (Qiagen) and DNA was eluted with water. Part of the purified DNA was used to perform a prosequencing PCR with the antisense primers to detect the SNP309 and to obtain a sequencing product, with 3 μ l of big dye terminator V3.1 cycle Sequencing kit (ABI, California, USA), 0.5 μ l of dimethyl sulfoxide (Sigma, Missouri, USA), 0.2 μ M of antisense primer and the eluted DNA under the following PCR conditions: 94°C (3 min), 32 cycles of 94°C (30 s), 51°C (60 s) and 60°C (3 min). Sequencing run was performed with the ABI Prism 377 DNA Sequencer machine. Sequences were aligned and checked for SNP309 via a DNA sequencing program, DNA SeqMan.

Genotyping was performed in batches of about 18–36 samples each time. Healthy and cancer samples were processed separately. Singaporean healthy samples were analyzed at the Singapore laboratory and the Shanghai samples (both cancer and healthy) were analyzed by the same investigator at the Shanghai laboratory. Cancer samples were analyzed in a blinded manner randomly, with no knowledge of the relevant information (i.e. onset age, status of the other genes such as *p53*, *MDM2*, etc.). All samples were numbered such as H1, H2 for healthy samples and C1, C2, etc. for cancer samples. To verify results, randomly selected samples (~10% of total) were reanalyzed by sequencing different individuals at both the laboratories, which gave consistent results throughout.

Statistical analysis

Fisher's exact test was used to evaluate Hardy–Weinberg equilibrium for the control healthy group. Odds ratio (OR) together with 95% confidence interval were estimated to investigate the role of SNPs in female breast cancer risk by means of unconditional logistic regression adjusted for age. The role of individual SNP in breast cancer was assessed appropriately based on the relationship of OR₁ (*p53*, Arg/Arg versus Pro/Pro; *MDM2*, G/G versus T/T), OR₂ (*p53*, Arg/Pro versus Pro/Pro; *MDM2*, G/T versus T/T) and OR₃ (*p53*, Arg/Arg versus Arg/Pro; *MDM2*, G/G versus G/T) to reflect a biological model of gene effect (33), as follows:

Recessive model: OR₁ = OR₃ \neq 1 and OR₂ = 1;
 Dominant model: OR₁ = OR₂ \neq 1 and OR₃ = 1;
 Over-dominant model: OR₂ = $\frac{1}{OR_3} \neq$ 1 and OR₁ = 1;
 Codominant model: OR₁ > OR₂ > 1 and OR₁ > OR₃ > 1 (or OR₁ < OR₂ < 1 and OR₁ < OR₃ < 1).

Kruskal–Wallis method was carried out to investigate the effects of SNPs on age of onset of disease, whereas Fisher's exact test was performed to evaluate the effects of SNPs on cancer stage and the comparison of the genotype distribution between healthy subjects and cases. All statistical analyses were performed using STATA version 9 (Stata Corporation, College Station, TX).

Results

Status of *MDM2* SNP309 in healthy Chinese population

The G/G genotype of *MDM2* SNP309 was found to be less frequently represented in healthy Caucasian populations, ranging from 1.7 to 16.3% compared with the T/T genotype (30,34–36). However, the G/G genotypic frequency was more abundant—up to 24%—in the healthy Ashkenazi Jewish population (30), who are generally thought to have a higher lifetime cancer risk (37). Consistently, some reports have noted an increase in the frequency of this genotype with accelerated age of onset in Caucasian populations, suggesting that this genotype could be associated with cancer risk (28,30). We therefore determined the prevalence of this SNP in the healthy Chinese population from two distinct geographical locations: Shanghai (China) and in Singapore (Singapore). The analysis indicated that the frequency of those with the G/G genotype was 21.4% ($n = 18$ of 84) in the Shanghai female population and much higher in the Singapore females (34.1% in females alone) ($n = 15$ of 44) (Table I). However, the difference between the two populations was not statistically significant ($P = 0.139$). Fisher's exact test showed that *MDM2* SNP309 polymorphism is in HWE among Shanghai healthy subjects ($P = 0.376$) and Singapore healthy subjects ($P = 1.000$). This higher

Table I. Status of *p53* codon 72 and *MDM2* SNP309 alleles in healthy Chinese population

Population	Genotype							
	<i>p53</i>				<i>MDM2</i>			
	<i>n</i>	Pro/Pro (%)	Pro/Arg (%)	Arg/Arg (%)	<i>n</i>	G/G (%)	G/T (%)	T/T (%)
Shanghai females	80	16.3	47.5	36.3	84	21.4	44.0	34.5
Jiangsu province females ^a	ND	ND	ND	ND	605	25.1	50.9	24.0
Singapore females	ND	ND	ND	ND	44	34.1	47.7	18.2

ND, not done.

^aData from Ma et al. (38).

incidence of the G/G genotypic frequency in the Chinese population correlated with that found in the Japanese population of which 33% was of this genotype (39) and another study on Chinese population from the Nanjing area in the Jiangsu province, which is on the south-east of China next to Shanghai (25.1%) (38). Comparison of the *MDM2* SNP309 frequencies between the Chinese population from Shanghai (this study) and the adjoining Jiangsu area (38) revealed no significant differences ($P = 0.130$) (Table I). It is also interesting to note that both the Japanese and Chinese populations have been reported to have a higher incidence of early onset non-familial breast cancers in contrast to the Caucasian population (6).

Comparison of the *p53* codon 72 genotype revealed that the Arg/Arg homozygotes were more prevalent compared with the Pro/Pro homozygotes in the Shanghai Chinese population (36.3–16.3%) ($n = 29/80$ – $13/80$) (Table I).

Breast cancer risk is associated with MDM2 SNP309 regardless of family history and the p53 Arg allele tended to be associated with reduced cancer risk in later onset cases

Peripheral blood samples from breast cancer patients of various ages were used to determine the role of the SNP309 and codon 72 polymorphism on cancer risk. All comparisons were made between healthy and cancer cases from Shanghai. We divided all the sporadic breast cancer patients into two groups, those <40 years of age and those >40 years of age and have termed them the early onset group (20–40 years of age) and later onset group (age of 41 and above). We have chosen this cutoff age as the earlier onset of the disease, which is non-familial, is seen in Asian Chinese women starting from 20 years of age, leading to a large number of cases occurring in women <40 years of age (5). Besides the sporadic cancers, 41 other cases had family history of cancers, with at least one first- or second-degree relative with breast and/or ovarian cancer, regardless of age (Table II). These are being referred to as familial breast cancer cases. Of these, only those of age <40 years had an association with *Brcal* mutations. Essentially, of the 32 cases <40 years of age, 6 had *Brcal* mutations and 1 had *Brcal2* mutation (data not shown). Generally, *Brcal* mutations were rare in the other cases and had no correlation with *p53* or the SNP309 status (data not shown). Distribution of cancer stage at presentation was different in the early onset and later onset cancer groups ($P = 0.001$) (Table II).

The genotype distributions of *p53* between all breast cancer cases (familial + sporadic) and healthy control group were not significantly different (percent of Arg/Arg versus Pro/Pro versus Arg/Pro: control/cases → 36.3/26.7 versus 16.3/22.4 versus 47.5/50.9) ($n = 80$ for healthy and 393 for cases: 29/105 versus 13/88 versus 38/200) ($P = 0.193$) (Figure 2A). No significant effect of *p53* on breast cancer risk was detected, with ORs of Arg/Arg versus Pro/Pro, Arg/Pro versus Pro/Pro and Arg/Arg versus Arg/Pro being 0.54 (0.25, 1.16), 0.71 (0.34, 1.46) and 0.76 (0.43, 1.36), respectively (Table III). When the sporadic cases alone were considered (i.e. those without family history), the distribution was not significantly different (percent of Arg/Arg versus Pro/Pro versus Arg/Pro: control/cases → 36.3/26.9 versus 16.3/22.1 versus 47.5/51.0) ($n = 80$ for healthy and 357 for sporadic cases: 29/96 versus 13/79 versus 38/182) ($P = 0.218$)

Table II. Summary of breast cancer patient status

Age group (years)	≤40, <i>n</i> = 241	≥41, <i>n</i> = 208
Family history of breast cancer ^a	32/241	9/208
<i>Brcal</i> mutation associated with family history	7/32 ^b	0/9
Tumour grade		
0	7	1
I	50	42
II	107	129
III	31	12
IV	4	1

^aDefined as with at least one first- or second-degree relative with breast and/or ovarian cancer, regardless of age.

^bSix had mutations in *Brcal* and one had mutation in *Brcal2*.

(Figure 2B). No significant effect of *p53* on sporadic breast cancer risk was detected, with ORs of Arg/Arg versus Pro/Pro, Arg/Pro versus Pro/Pro and Arg/Arg versus Arg/Pro being 0.54 (0.25, 1.16), 0.71 (0.34, 1.46) and 0.76 (0.43, 1.36), respectively (Table III). Moreover, of the 35 cases with family history of breast cancer and were genotyped, 9 were Arg/Arg (25.7%), 17 were Arg/Pro (48.6%) and 9 (25.7%) were of the Pro/Pro genotype. This distribution was not significantly different from that in healthy subjects ($P = 0.368$) (Figure 2C).

However, there was a trend of codominant effect of *p53* polymorphism on breast cancer risk >40 years of age in the sporadic cases, with estimated ORs of Arg/Arg versus Pro/Pro, Arg/Pro versus Pro/Pro and Arg/Arg versus Arg/Pro of 0.26 (0.07, 0.90), 0.52 (0.16, 1.75) and 0.49 (0.19, 1.23), respectively (Table III). Together, these results indicate that although the *p53* codon 72 SNP does not affect general breast cancer risk in the Chinese population, the Arg variant might decrease sporadic breast cancer risk via a codominant mode in the later onset group.

In contrast to *p53*, the frequency of the *MDM2* SNP309 G/G homozygotes was significantly increased in breast cancer cases (familial + sporadic), concomitant with a decrease in the T/T homozygotes (percent of G/G versus T/T versus G/T: healthy control/cases → 21.4/30.6 versus 34.5/18.7 versus 44.0/50.7) ($n = 84$ for healthy and 402 for cases: 18/123 versus 29/75 versus 37/204) ($P = 0.006$) (Figure 2A). The ORs of G/G versus T/T, G/T versus T/T and G/G versus G/T were 2.92 (1.46, 5.85), 2.56 (1.41, 4.66) and 1.14 (0.60, 2.14), respectively, which suggested that the variant G allele is associated with increased breast cancer risk in a dominant mode [G/T + G/G versus T/T: 2.68 (1.54, 4.69)] (Table IV). Comparison of the cancer frequencies from our study with the healthy control frequencies obtained from the larger study from the Jiangsu area also revealed that the G/G homozygotes were increased in breast cancer cases (percent of G/G versus T/T versus G/T: healthy control/cases → 25.1/30.6 versus 24.0/18.7 versus 50.9/50.7) ($n = 605$ for healthy and 402 for cases: 152/123 versus 145/75 versus 308/204) ($P = 0.055$) (Figure 2A) as the breast cancer risk was increased among subjects with G/G either for all females [G/G versus T/T: 1.56 (1.08, 2.26)].

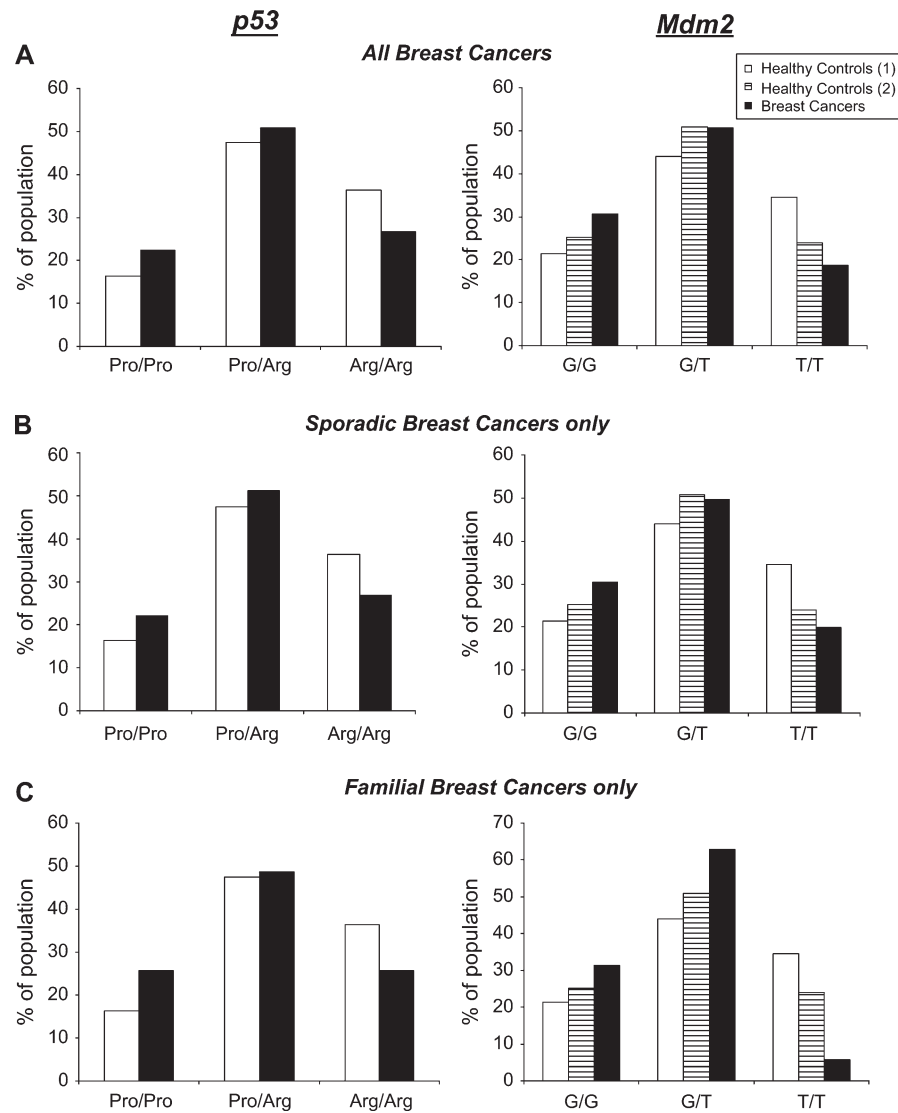


Fig. 2. *MDM2* SNP309, but not codon 72 polymorphism of *p53*, affects breast cancer risk (A–C). Genotypic analysis of *p53* codon 72 polymorphism (left panel) and SNP309 of *MDM2* (right panel) comparing the frequency of various genotypes in healthy controls and breast cancer cases, analyzed as all cases (A), sporadic cases only (B) and familial cases only (C). $n = 84$ (*Mdm2*) and $n = 80$ (*p53*) for healthy samples from this study, designated as healthy controls (1), and $n = 605$, for healthy samples from a related study by Ma *et al.* (38), designated as healthy controls (2). $n = 393$ (*p53*) and $n = 402$ (*Mdm2*) for all breast cancer cases, and $n = 35$ for both *p53* and *MDM2* for familial breast cancer cases.

Table III. The effects of *p53* codon 72 SNP on female breast cancer risk

	OR (95% confidence interval) ^a		
	Arg/Pro versus Pro/Pro	Arg/Arg versus Pro/Pro	Arg/Arg versus Arg/Pro
Sporadic later onset (>40)	0.52 (0.16, 1.75)	0.26 (0.07, 0.90)	0.49 (0.19, 1.23)
Sporadic early onset ^b (≤40)	0.80 (0.27, 2.38)	0.80 (0.24, 2.69)	1.00 (0.39, 2.53)
Sporadic later onset (>50)	0.55 (0.17, 1.77)	0.27 (0.08, 0.93)	0.50 (0.20, 1.24)
Sporadic early onset ^b (≤50)	0.84 (0.28, 2.49)	0.82 (0.24, 2.77)	0.98 (0.38, 2.50)
All sporadic cases (non-familial)	0.71 (0.34, 1.46)	0.54 (0.25, 1.16)	0.76 (0.43, 1.36)
Familial cases (non-sporadic) ^c	0.64 (0.20, 2.02)	0.55 (0.15, 1.96)	0.87 (0.30, 2.48)
All cases (sporadic + familial)	0.71 (0.34, 1.47)	0.54 (0.25, 1.16)	0.76 (0.43, 1.36)

^aOR and corresponding confidence interval were adjusted for age.

^bSporadic early onset patients who do not have familial history of cancer.

^cDefined as with at least one first- or second-degree relative with breast and/or ovarian cancer, regardless of age.

Table IV. The effects of *MDM2* SNP309 on female breast cancer risk

	OR (95% confidence interval) ^a			
	G/T versus T/T	G/G versus T/T	G/G versus G/T	G/T + G/G versus T/T
Sporadic later onset (>40)	3.02 (1.12, 8.11)	4.78 (1.49, 15.33)	1.58 (0.58, 4.36)	3.52 (1.38, 8.99)
Sporadic early onset ^b (≤40)	1.68 (0.68, 4.17)	1.32 (0.50, 3.54)	0.79 (0.31, 2.03)	1.53 (0.68, 3.45)
Sporadic later onset (>50)	2.88 (1.07, 7.74)	4.81 (1.52, 15.15)	1.67 (0.62, 4.50)	3.45 (1.36, 8.78)
Sporadic early onset (≤50)	1.72 (0.69, 4.29)	1.29 (0.48, 3.47)	0.75 (0.29, 1.93)	1.53 (0.68, 3.49)
All sporadic cases (non-familial)	2.33 (1.28, 4.24)	2.81 (1.40, 5.63)	1.21 (0.64, 2.28)	2.49 (1.43, 4.35)
Familial cases (non-sporadic) ^c	11.46 (2.26, 58.14)	6.78 (1.25, 36.81)	0.59 (0.21, 1.70)	9.32 (1.96, 44.45)
All cases (sporadic + familial)	2.56 (1.41, 4.66)	2.92 (1.46, 5.85)	1.14 (0.60, 2.14)	2.68 (1.54, 4.69)

^aOR and corresponding confidence interval were adjusted for age.

^bSporadic early onset patients who do not have familial history of cancer.

^cDefined as with at least one first- or second-degree relative with breast and/or ovarian cancer, regardless of age.

A similar trend to the total cases was also noted when only the sporadic breast cancer cases were compared with our matched healthy controls (percent of G/G versus T/T versus G/T: healthy control/cases → 21.4/30.5 versus 34.5/19.9 versus 44.1/49.6) ($n = 84$ for healthy and 367 for cases: 18/112 versus 29/73 versus 37/182) ($P = 0.015$) (Figure 2B), with the ORs of G/G versus T/T, G/T versus T/T and G/G versus G/T being 2.81 (1.40, 5.63), 2.33 (1.28, 4.24) and 1.21 (0.64, 2.28), respectively, which suggested that the variant G allele is associated with increased breast cancer risk in a dominant mode [G/T + G/G versus T/T: 2.49 (1.43, 4.35)] (Table IV). Interestingly, the variant G allele increased breast cancer risk in a dominant mode, especially among females >40 years of age [G/T + G/G versus T/T: 3.52 (1.38, 8.99)] (Table IV). Comparison with the Jiangsu controls also revealed that the sporadic breast cancer risk (non-familial) was increased among subjects with G/G [G/G versus T/T: 1.46 (1.01, 2.12)].

Of the 35 cases with family history and genotyped, 11 were of the G/G (31.4%), 22 were G/T (62.9%) and only 2 (5.7%) were of the T/T genotype, which was significantly different from the genotype distribution of controls ($P = 0.002$) (Figure 2B). The ORs of G/G versus T/T, G/T versus T/T and G/G versus G/T were 6.78 (1.25, 36.81), 11.46 (2.26, 58.14) and 0.59 (0.21, 1.70), respectively, implying a dominant effect of variant G [G/T + G/G versus T/T: 9.32 (1.96, 44.45)] (Table IV). Comparison of the familial cancer frequencies with the healthy control frequencies obtained from the larger study from the Jiangsu area also revealed a similar trend (percent of G/G versus T/T versus G/T: healthy control/cases → 25.1/31.4 versus 24.0/5.7 versus 50.9/62.9) ($P = 0.027$) (Figure 2C), with a dominant effect of variant G [G/T + G/G versus T/T: 5.20 (0.23, 21.90)]. Together, these results indicate a positive correlation between the G allele of *MDM2* SNP309 with breast cancer risk, both among females >40 years of age with sporadic cancers and those with family history of cancer.

Altering the cutoff age to <50 or >50 did not change the conclusions for both *p53* and *MDM2* status, as the ORs were similar to that obtained using 40 years as a cutoff age (Tables III and IV).

p53 codon 72 polymorphism has no effect on age of onset or stage of breast cancer

We next evaluated if *p53* polymorphism at codon 72 affected the age of disease onset. Kruskal–Wallis analysis indicated no association of any of the *p53* alleles with age of onset ($P = 0.806$) (Table V and Figure 3A). The mean age of onset for the Arg/Arg, Arg/Pro and Pro/Pro groups were found to be 43.14 ± 13.28, 42.04 ± 12.56 and 43.02 ± 13.28, respectively. Moreover, there was no effect of the various genotypes on the age of onset either among sporadic cases ($P = 0.721$) (Figure 3B) or familial cases ($P = 0.942$) (Figure 3C). Similarly, Fisher's exact test indicated no association of any of the *p53* alleles with the stage of breast cancers ($P = 0.652$) (Table V and supplementary Figure 1A, available at *Carcinogenesis* Online). All genotypes were equally represented in all stages of the disease (supplementary Figure 1A, available at *Carcinogenesis* Online).

Table V. *P* values of the effects of *p53* codon 72 and/or *MDM2* SNP309 on the age onset and cancer stage of female breast cancer

	<i>p53</i>	<i>MDM2</i>
Age onset	0.806	0.033
Cancer stage	0.652	0.610

MDM2 SNP309 G allele delays onset of breast cancer but does not affect stage of cancer

As the *MDM2* SNP309 G allele was suggested in some studies to be associated with earlier onset age of cancers in females (30), we analyzed if this phenomenon is applicable to our study population where early onset of the disease is a common feature. Kruskal–Wallis analysis revealed that SNP309 indeed affected the onset age of breast cancer patients (sporadic + familial) ($P = 0.033$) (Table V). The mean age of onset for the G/G, G/T and T/T groups were found to be 44.11 ± 13.70, 44.46 ± 13.65 and 39.99 ± 12.26, respectively. Pair-wise test of SNP309 showed that patients with G/T genotype tend to develop cancer significantly later than patients with T/T genotype ($P = 0.011$) (Figure 3A). Moreover, patients with G/G genotype also developed cancers slightly later in life compared with those with the T/T genotype ($P = 0.03$). Whereas ~61% of those with the T/T genotype had developed breast cancer by 40 years of age, only 47% of G/T and 50% of G/G genotypes had the disease by this age (Figure 3A). The difference between those with the G/T and G/G genotypes was not significant ($P = 0.841$) (Figure 3A). Similarly, when the rate of cancer onset was analyzed based on 50% of the population for each genotype, the average age was 34 years for those with the T/T genotype, whereas it was 42 and 39 years for those with the G/T and G/G genotypes, respectively (Figure 3A).

Further analysis of sporadic cases only also showed a similar trend (Figure 3B). The mean age of onset for the G/G, G/T and T/T groups were found to be 45.33 ± 13.73, 44.76 ± 13.94 and 40.23 ± 12.32, respectively. Pair-wise test of SNP309 also showed that patients with G/T genotype tend to develop cancer significantly later than patients with T/T genotype ($P = 0.020$) (Figure 3B). Moreover, patients with G/G genotype also developed cancers later in life compared with those with the T/T genotype ($P = 0.009$). Whereas ~61% of those with the T/T genotype had developed breast cancer by 40 years of age, only 45% of both G/T and G/G genotypes had the disease by this age (Figure 3B). The difference between those with the G/T and G/G genotypes was not significant ($P = 0.595$) (Figure 3B). Similarly, when the rate of cancer onset was analyzed based on 50% of the population for each genotype, the average age was 34 years for those with the T/T genotype, whereas it was ~46.5 years for those with the G/T and G/G genotypes (Figure 3B).

In contrast, analysis of familial cancers only revealed that those with G/T genotypes were found to develop cancer later than those

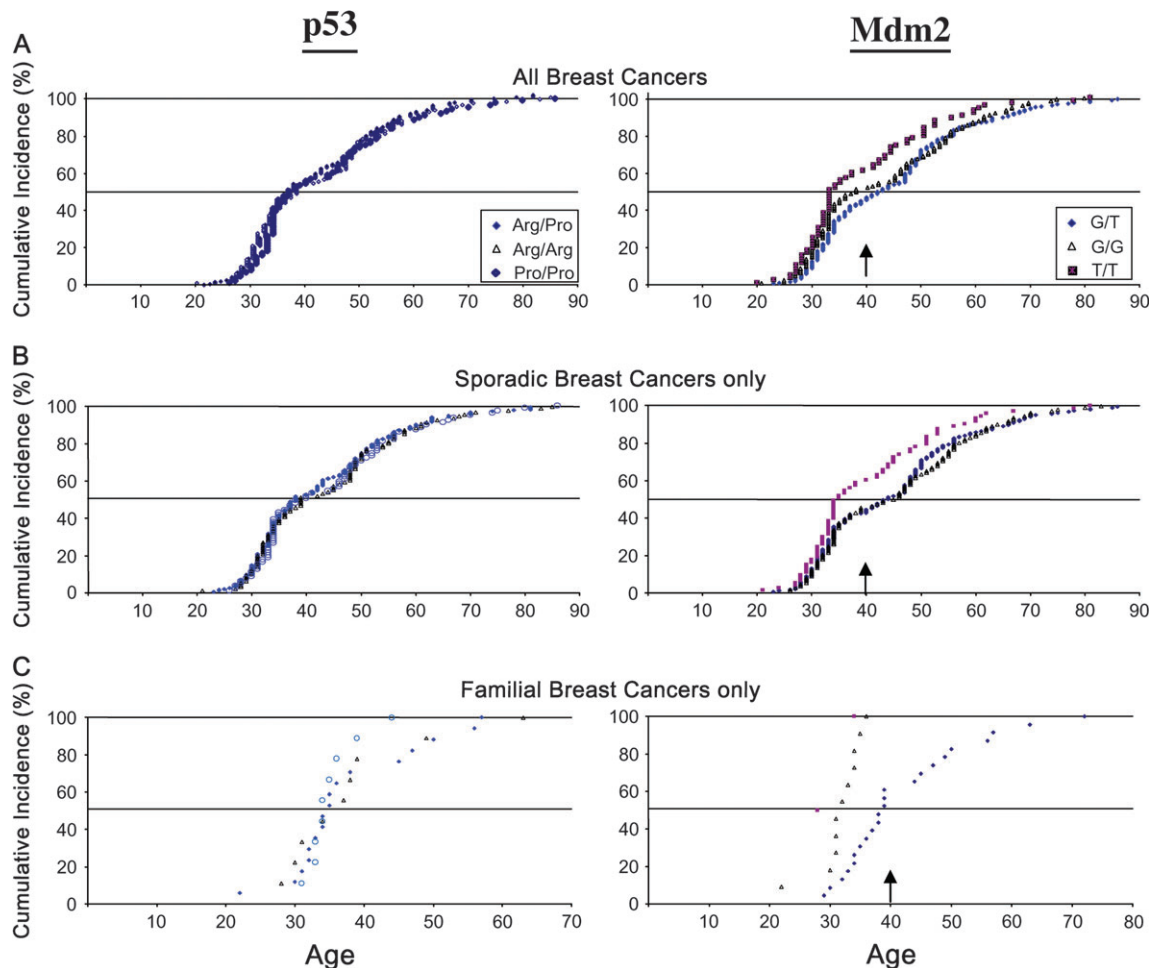


Fig. 3. *MDM2* SNP309 T allele is associated with accelerated age of onset of breast cancers, but *p53* polymorphism does not affect age of onset. (A–C) The cumulative incidence of cancer cases in the population was plotted as a function of age of onset of breast cancer in females. No significant differences were noted with respect to the different *p53* codon 72 polymorphic genotypes. $n = 88$ for Pro/Pro, $n = 105$ for Arg/Arg and $n = 200$ for Arg/Pro for all cases (A) (left panel). In contrast, the T/T allele of the *MDM2* SNP309 is enriched in younger women, especially in the sporadic breast cancer cases (A–B). Women with the G/G and G/T genotypes were found to have later onset of breast cancer in sporadic cases. $n = 75$ for T/T, $n = 123$ for G/G and $n = 204$ for G/T in all cases (A), $n = 73$ for T/T, $n = 112$ for G/G and $n = 181$ for G/T in sporadic cancers only (B) and $n = 2$ for T/T, $n = 11$ for G/G and $n = 23$ for G/T in familial cancers only (C). Arrow indicates 40 years of age.

with G/G genotype (42.32 ± 11.32 versus 31.73 ± 3.74) ($P = 0.001$) (Figure 3C), a finding that is consistent with previous studies highlighting that the presence of the T allele can delay the onset of familial cancers in females.

Analysis of the effects of SNP309 on stage of disease did not reveal any significant differences among the different genotypes and the stages of breast cancer ($P = 0.610$) (Table V and supplementary Figure 1B, available at *Carcinogenesis* Online). All genotypes were equally represented in all stages of the disease (supplementary Figure 1B, available at *Carcinogenesis* Online).

Together, the results suggest that although SNP309 did not affect the stage of cancer, those with the T/T genotype tended to be more prone to earlier onset of sporadic breast cancer in the Chinese population.

Discussion

The data presented here suggest that the G allele of *MDM2* SNP309 increases both sporadic and familial breast cancer risk. However, unexpectedly, the T allele in contrast to the G allele was found to be associated with earlier onset age of sporadic breast cancers in the Chinese population. In addition, although *p53* codon 72 did neither affect onset age of the disease nor cancer risk generally, the Arg allele

was associated with a tendency to decrease sporadic breast cancer risk in older women.

We undertook this study to evaluate if the relatively higher incidence of early onset non-familial breast cancers that is uniquely observed in the Asian Chinese population could be due to the effects of the SNPs in the *MDM2/p53* axis. Interestingly, this population contains a higher frequency of the G allele of *MDM2* SNP309 (this report and ref. 38), which has been suggested to lead to higher transcription and hence, higher levels of *MDM2* (28). However, though the SNP309 G allele seemed to increase breast cancer risk in the Asian Chinese population, it was not associated with early onset of the disease in sporadic cases. In fact, the G allele delayed the onset age, whereas the T allele was associated with earlier onset of the disease. These data are unexpected based on the earlier studies, as well as our findings with familial cases (Figure 3C) that suggest a positive correlation between the G allele and an earlier age of female breast cancer onset.

With the initial identification of the SNP in *MDM2* promoter, there has been a series of investigations aimed at validating the role of SNP309 G allele with early onset age of cancers. Since the SNP was noted in the SP-1-binding motif in the *MDM2* promoter where ER was shown to bind (40,41), it has been suggested to affect estrogen signaling, and hence, affected by estrogen levels (30). Consistently,

the distinct effects of this SNP were noted in females in contrast to males (30).

Closer analysis of the published data, however, reveals that there may not be a straight correlation between the SNP309 G allele and age of breast cancer onset. Bond *et al.* (28,30) in their first two reports found a correlation between this G allele and earlier onset of female breast cancers, particularly in familial Li–Fraumeni cases. Supporting these data, a recent report also suggested that the G allele was associated with accelerated onset of familial breast cancers, but concluded that this had no association with estrogen signaling (42). In contrast, several other studies investigating breast cancers did not observe any association between the G allele and age of cancer onset (34,35,38,43). The major difference between the limited studies that support a positive correlation between the G allele and breast cancer onset age and those that did not find any correlation is that the earlier studies analyzed familial cancer cases, whereas the latter studies evaluated sporadic cancers. Thus, it appears that the G allele is probably associated with early age of onset in familial cancer cases, an observation that we could confirm, as those with G/G genotype tended to develop cancer earlier than those with G/T genotype (Figure 3C). This could be taken to mean that there may be other factors that would prime these familial cases to cancer predisposition, perhaps in an estrogen-dependent manner. On the contrary, sporadic cases without any form of underlying familial genetic risk may not have this priming factor that would enable the manifestation of the effects of the G allele (which increases MDM2 levels). Thus, one could envisage that in familial cases where the p53 pathway is already often affected and weakened, increasing the MDM2 levels (by the presence of the G allele) would enhance cancer susceptibility probably through the alteration of other gene products that suppress tumorigenesis. In sporadic cases, however, where the p53 pathway may not be affected yet, presence of the G allele alone would not be sufficient to accelerate the age of cancer onset as MDM2 could be a limiting factor in inhibiting p53 and other potential tumor suppressors. Further investigations are required to test this hypothesis and to identify other molecular targets of MDM2 that may be responsible for the early onset of cancers in familial cases.

The most important finding presented here is that instead of the initially expected association of the G allele with early age of cancer onset noted in familial cancer cases, we observed a significant association between the T/T genotype and earlier onset age in sporadic cancer cases. It is also interesting to note that when Bond *et al.* (30) segregated familial breast cancer patients based on ER positivity, the ER patients with the T/T genotype had an earlier age of cancer onset compared with those who were of the G/G genotype. Though we do not have ER data to correlate with the MDM2 genotypes, it is evident that T/T genotype can also be associated with promotion of the early onset of female cancers, though the molecular details are not known. These findings cannot be explained merely based on the elevated estrogen signaling in younger women. Moreover, it is also conceivable that the T allele, with the expected lowered MDM2 production, could promote the early onset of cancers through modulating the p53 pathway. Hence, it is possible that the T allele could affect the recruitment of different factors onto the MDM2 promoter, thereby influencing its transcription, therefore suggesting that MDM2 may have a p53-dependent and -independent effect with respect to familial and sporadic breast cancer cases, respectively. Altogether, these data suggest that the G allele need not always be associated with earlier onset of cancers, as was shown in familial cases, but the opposite could be true in sporadic cases.

Besides this difference, our study also revealed that there is an association of the G allele with breast cancer risk in Asian Chinese females. It is noteworthy that the Chinese (and Japanese) population contains a higher frequency of people with the G genotype (this report and refs 37,38), which correlates with a higher percentage of them being predisposed to early onset of sporadic breast cancers (6). However, there have been no reports associating the G allele with general breast cancer risk in other populations (34,35,38,43). Moreover, the age-adjusted ratio of general cancer risk is generally lower in the

Chinese (Asians) than in the western Caucasian populations (1), arguing against a general mechanism for the MDM2 SNP309 G allele to be associated with pan-cancer risk. Therefore, it is possible that the G allele can affect breast cancer risk in the Chinese population, perhaps in combination with estrogen levels and with other ethnicity-associated genetic factors that have yet to be identified.

Finally, although the status of MDM2 SNP309 is correlated with cancer risk and age of onset, the p53 codon 72 polymorphism, that has functional significance, did not seem to affect age of cancer onset. This suggests that MDM2 may be targeting other substrates besides p53 in influencing cancer predisposition. Nonetheless, consistent with the notion that the p53 pathway is often affected in cancers, this p53 polymorphism was indeed found to decrease the risk of sporadic breast cancers. The Arg variant was found to decrease the risk compared with the Pro form, especially in older women. This indicates that the functionality of the p53 polymorphism may be relevant in influencing cancer predisposition, besides its effects on survival and prognosis when the gene is mutated (10,12).

Taken together, the data presented here suggest that there is a positive correlation between the G allele of MDM2 SNP309 and breast cancer risk in the Asian Chinese population. Moreover, the T/T genotype, which is thought to lead to lowered production of MDM2, is associated with the earlier onset of non-familial breast cancer cases. This is in contrast to the positive correlation of the SNP309 G allele with accelerated age of onset of familial breast cancer cases and other cancers such as gastric and nasopharyngeal carcinoma (44,45). These findings therefore demonstrate that MDM2 SNP309 indeed affects cancer risk and age of onset, but in different ways in the Asian Chinese population, highlighting that other ethnicity-associated factors may also have essential roles in determining cancer susceptibility.

Supplementary material

Supplementary Figure 1A and B can be found at <http://carcin.oxfordjournals.org/>

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