Investigation of the Effect of *MDM2* SNP309 and *TP53* Arg72Pro Polymorphisms on the Age of Onset of Cutaneous Melanoma

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Melanoma accounts for the majority of deaths from skin cancer. Women tend to be diagnosed at a younger age and have better survival than men. A tumor–host interaction might be responsible for these gender-specific differences. Recently, a functional single-nucleotide polymorphism in the promoter of the *human homolog of mouse double minute 2* (*MDM2*) gene was characterized: single-nucleotide polymorphism (SNP)309 increases the *MDM2* transcription. In melanoma, the effects for SNP309 and the related tumor protein p53 (TP53) Arg72Pro are inconsistent among published reports. This study investigated the association between SNP309 (RefSNP accession ID (rs)2279744) and *TP53* codon 72 (rs1042522) polymorphisms, with outcome in a hospital-based cohort of 990 patients with melanoma. We assessed whether these polymorphisms were associated with clinicopathological and phenotypic characteristics and whether these SNPs affect the age of onset of the disease, recurrence, and survival. No significant associations were found between the SNPs and survival. However, women carrying the SNP309 GG genotype were less likely to be diagnosed at a younger age: odds ratio$_{\text{adjusted}<50}$ 0.52 (0.29–0.92). Our results suggest that women carrying the SNP309 GG genotype might be at lower risk of developing melanoma at a younger age compared with those carrying TG or TT. Further studies are needed to determine whether a nearby functional polymorphism is responsible for this effect in premenopausal women.

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INTRODUCTION

Melanoma incidence continues to rise and accounts for the great majority of deaths from skin cancer, with an estimated 8,700 deaths in 2010 (Jemal *et al.*, 2010). The incidence of melanoma is higher among women during the reproductive years, but after the age of 50 years this cancer is more frequent among men (Horner *et al.*, 2009). Although women tend to be diagnosed at an earlier age, they have better survival than men (de Vries *et al.*, 2008; Jemal *et al.*, 2010). Factors other than stage at diagnosis and body site seem to be responsible for these gender differences, and a tumor–host interaction has been proposed (de Vries *et al.*, 2008; Lasithiotakis *et al.*, 2008; Joosse *et al.*, 2011).

A frequent single-nucleotide polymorphism (SNP) in the human *homolog of mouse double minute 2* (*MDM2*) gene, commonly referred to as SNP309, was found to be a genetic risk modifier of cancer incidence in individuals with the Li–Fraumeni syndrome (Bond *et al.*, 2004). The SNP consists of a T to G change, and the authors proposed that the G allele favors the binding of the specific protein 1 transcription factor, which results in elevated levels of *MDM2* mRNA and protein, thereby attenuating the p53 response. In addition, it has been proposed that SNP309 regulates *MDM2* expression through enhanced binding of the specific protein 1 transcription factor and estrogen receptor (Okumura *et al.*, 2002; Kinyamu and Archer, 2003; Bond *et al.*, 2006a). Some studies, but not all, reported that the *MDM2* SNP309 might modify the risk, age of onset, or prognosis in a variety of cancers (Alhopuro *et al.*, 2005; Bond *et al.*, 2006b; Schmidt *et al.*, 2007; Wilkening *et al.*, 2007; Krekac *et al.*, 2008; Fang *et al.*, 2010; Yu *et al.*, 2011). In melanoma, there are only a few investigations on *MDM2* SNP309 to date, and the reported effect for this SNP is not consistent between studies: one study found an association between SNP309 and risk for melanoma, as well as age at diagnosis, and other studies found no effect on either risk, age at diagnosis, or progression

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Abbreviations: Arg, arginine; CI, confidence interval; MDM2, human homolog of mouse double minute 2; OR, odds ratio; Pro, proline; rs, RefSNP accession ID; SNP, single-nucleotide polymorphism; TP53, tumor protein p53

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(Firoz *et al.*, 2009; Gluck *et al.*, 2009; Nan *et al.*, 2009; Capasso *et al.*, 2010).

Similarly, the effect of a second widely studied polymorphism in the Mdm2–p53 pathway varies across different studies. The *TP53* polymorphism on codon 72 consists of a G-to-C transition that results in the substitution of arginine (Arg) to proline (Pro) (Matlashewski *et al.*, 1987). It has been proposed that the status of codon 72 can influence the biological behavior of some p53 mutants (Marin *et al.*, 2000). The allelic variants exhibit distinct structural features and biological properties, with the codon 72 Pro being credited with a more efficient transcriptional activation and suppression of cell growth, and the arginine with a less efficient induction of apoptosis (Thomas *et al.*, 1999). With regard to melanoma, two epidemiological studies reported an increased risk associated with the genotype Arg/Arg (Shen *et al.*, 2003; Li *et al.*, 2008), two studies found an association with the genotype Pro/Pro (Gwosdz *et al.*, 2006; Stefanaki *et al.*, 2007), and other studies found no effect with the development or progression of melanoma (Povey *et al.*, 2007; Firoz *et al.*, 2009). Some of the inconsistencies found across the different studies may be due to differences in sample size, study design, or characteristics of the study subjects.

In this hospital-based study, we genotyped the *TP53* codon 72 (G>C, RefSNP accession ID (rs)1042522) and the *MDM2* SNP309 (T>G, rs2279744) polymorphisms in a cohort of 990 individuals with melanoma and assessed whether these polymorphisms are associated with the patients' clinical, pathological, and phenotypic characteristics and whether these SNPs affect the age of onset of the disease, recurrence, and survival.

RESULTS

The characteristics of the study participants are described in Table 1. In all, 96% were non-Hispanic white and 4% were Hispanic, non-Hispanic black, and Asian/Indian. We found that women were diagnosed 7 years (median) earlier than men, tended to have lighter skin, and their tumors were diagnosed at lower stages, with smaller Breslow thickness values and lower Clark level. Furthermore, their melanomas occurred on the extremities more often than in men. Age at diagnosis, thickness, stage at diagnosis, phenotypic index, and anatomic site were significantly associated with gender after correcting for multiple testing. There were 195 deaths, including 113 disease-specific deaths, and 285 recurrences. The mean follow-up for the survivors was 6 years. The genotype and allele frequencies are shown in Table 2.

Effect of *MDM2* and *TP53* genotypes on recurrence and survival

MDM2 SNP309 G allele did not have a significant effect on the risk of death of melanoma or on disease recurrence, after adjusting for covariates (Tables 3 and Supplementary Table S1 online). The results were similar when the analysis was restricted to non-Hispanic whites. We did not find any significant associations between Arg72Pro (G>C, rs1042522) and disease recurrence or survival (Table 3).

Table 1. Characteristics of the study participants

	Total, *N* (%)	Women, *N* (%)	Men, *N* (%)	*P*-value[1]
Total cases	990 (100)	421 (42.5)	569 (57.5)	
Age at diagnosis (median years and range)	55.2 (5–89)	50.1 (5–88)	57.2 (10–89)	**<0.01**
Race				0.89
Non-Hispanic white	951 (96.1)	404 (95.9)	547 (96.1)	
Other[2]	39 (3.9)	17 (4.1)	22 (3.9)	
Breslow thickness (median, mm)	1.5	1.2	1.5	**<0.01**
Stage at diagnosis				**0.01**
0	69 (7.0)	34 (8.2)	35 (6.2)	
I	492 (49.7)	227 (54.4)	265 (47.2)	
II	219 (22.2)	90 (21.6)	129 (22.9)	
⩾III	199 (20.1)	66 (15.8)	133 (23.7)	
Missing	11 (1.1)			
Clark level				**0.04**
I	58 (5.9)	30 (8.0)	28 (5.6)	
II	106 (10.7)	51 (13.6)	55 (11.0)	
III	150 (15.2)	72 (19.3)	78 (15.6)	
IV	490 (49.5)	200 (53.5)	290 (58.2)	
V	69 (7.0)	21 (5.6)	48 (9.6)	
Missing	117 (11.8)			
Multiple primary melanoma	175 (17.7)	70 (16.6)	105 (18.5)	0.45
Family history of melanoma	175 (17.7)	83 (19.7)	92 (16.2)	0.15
Phenotypic index[3]				**0.02**
1	107 (10.8)	48 (11.7)	59 (10.6)	
2	194 (19.6)	79 (19.2)	115 (20.7)	
3	366 (37.0)	138 (32.6)	228 (41.1)	
4	222 (22.4)	102 (24.8)	120 (21.6)	
5	78 (7.9)	44 (10.7)	34 (6.1)	
Missing	23 (2.3)			
Nevi				0.84
None	259 (26.2)	105 (26.1)	154 (27.8)	
Few	492 (49.7)	211 (52.4)	281 (50.6)	
Moderate	167 (16.9)	72 (17.9)	95 (17.1)	
Many	40 (4.0)	15 (3.7)	25 (3.7)	
Missing	32 (3.2)			
Anatomic site				**<0.01**
Head/face	72 (7.3)	18 (4.3)	54 (9.5)	
Trunk	339 (34.2)	101 (24.0)	238 (41.8)	
Extremity	537 (54.2)	286 (67.9)	251 (44.1)	
Unknown	42 (4.2)	16 (3.8)	26 (4.6)	

[1]*P*-values were calculated with χ^2 test for gender; significant *P*-values (⩽0.05) appear in bold type.
[2]Hispanic, black non-Hispanic, and Asian/Indian.
[3]Defined in the Materials and Methods section.

Table 2. *TP53* codon 72 (Arg/Pro) and *MDM2* SNP309 genotype and allele distributions

	Reported frequency (%)[1,2]	Total, *N* (%)	Women, *N* (%)	Men, *N* (%)
MDM2 SNP309 (rs2279744)				
TT	36.5–45.7	360 (36.5)	147 (35.2)	213 (37.5)
TG	41.8–47.5	451 (45.7)	194 (46.4)	257 (45.2)
GG	12.2–19.2	175 (17.8)	77 (18.4)	98 (17.3)
T	61.8–63.4	1,171 (59.4)	488 (58.4)	683 (60.1)
G	36.6–38.2	801 (40.6)	348 (41.6)	453 (39.9)
Total		986 (100)	418 (100)	568 (100)
TP53 c.72 (rs1042522)				
GG (Arg/Arg)	58–62	524 (54.0)	220 (53.3)	304 (54.5)
GC (Arg/Pro)	29–30	374 (38.5)	164 (39.7)	210 (37.6)
CC (Pro/Pro)	8.3–12.9	73 (7.5)	29 (7.0)	44 (7.9)
G (Arg)	77	1,422 (73.2)	604 (73.1)	818 (73.3)
C (Pro)	23	520 (26.8)	222 (26.9)	298 (26.7)
Total		971 (100)	413 (100)	558 (100)

Abbreviations: Arg, arginine; dbSNP, single-nucleotide polymorphism database; MDM2, human homolog of mouse double minute 2; Pro, proline; rs, RefSNP accession ID; TP53, tumor protein p53.
[1]Genotype and allelic frequencies obtained for TP53 codon 72 (rs1042522) in dbSNP, accessed in January 2011 (Caucasian and HapMap-Caucasian male).
[2]Frequencies for *MDM2* SNP309 (rs2279744) are not available in dbSNP and represent a range of those reported in cancer-free controls from the United Kingdom, United States, and Italy (Schmidt *et al.*, 2007; Ellis *et al.*, 2008; Nan *et al.*, 2009; Capasso *et al.*, 2010).

Effect of MDM2 and TP53 genotypes on clinicopathological and phenotypic features

It has been proposed that the effect of the SNP309 occurs in a gender-specific and hormone-dependent manner. Therefore, we conducted a stratified analysis by gender and age. We found that women carrying one or two Gs were diagnosed at an older age (mean: 51.7 and 54.3 years old; median: 50.1 and 57.6 years old, respectively) than those carrying two Ts (mean: 48.3 years old; median 48.6 years old), and that this difference is significant ($P=0.03$) (Table 4), although not when the *P*-value is adjusted for multiple comparisons. When stratified by decade of age, this difference was of borderline significance (Supplementary Table S2 online). Figure 1a shows the distribution of age at diagnosis by SNP309 genotype for men and women. We then calculated the odds ratios (OR) and 95% confidence intervals (95% CI) using logistic regression. For these analyses, we dichotomized the variable ''age at diagnosis''. We used varying age cutoffs in order to calculate the odds of being diagnosed with melanoma by a certain specified age for patients carrying the SNP309 GG compared with TG or TT genotypes (Table 5). In univariate analysis, we found that women homozygous for the G allele are at lower risk of developing melanoma within the <50 and <60 age groups. After adjusting for tumor thickness, stage, and family history, the GG genotype appeared protective in younger women ($OR_{<50vs.>50}$ 0.60, 95% CI 0.35–1.00, $P=0.05$; Table 5). Very similar results were obtained when analysis was restricted to white Caucasians ($OR_{<60vs.>60}$ 0.53, 95% CI 0.31–0.90, $P=0.02$), and when we excluded those with *in situ* lesions ($OR_{<60vs.>60}$ 0.55, 95% CI 0.31–0.94, $P=0.03$). The results remained statistically significant after including phenotypic index and moles in the model ($OR_{<50vs.>50}$ 0.52, 95% CI 0.29–0.92, $P=0.02$; Table 5), and after correcting for multiple testing ($P=0.05$). We did not see the same effect among men.

We did not find any associations between Arg72Pro (G>C, rs1042522) and patients' clinicopathological or phenotypic characteristics (Supplementary Table S3 online and Figure 1b). We also combined the two polymorphisms, *MDM2* SNP309 and *TP53* codon 72, and found no significant differences in the distribution of TP53 genotypes according to *MDM2* genotypes in either women or men (data not shown).

DISCUSSION

This study evaluated the associations between the polymorphisms *MDM2* SNP309 T>G and *TP53* codon 72 Arg>Pro on the clinicopathological and phenotypic characteristics, age of onset, recurrence, and survival in a cohort of 990 patients with melanoma. This study does not support the GG genotype as a risk factor for early onset of melanoma in women. Our results suggest that women carrying the GG genotype might be at a lower risk of developing melanoma under the age of 60 years than those carrying TG or TT.

The rate of somatic *TP53* mutations in melanoma is very low; however, p53 has a central role in pigmentation and tanning response, and there is a myriad of mechanisms by which this tumor suppressor's pathway may be deregulated in tumors (Box and Terzian, 2008). Thus, several studies investigated the association between tumor features and the only known common non-synonymous SNP in *TP53* (codon 72); however, the results are still controversial. With regard to melanoma, Li *et al.* (2008), in an extension of their initial investigation (Shen *et al.*, 2003), found a positive association between the GG (Arg/Arg) genotypes and risk, whereas two other studies found an increased risk of melanoma for carriers of the codon 72 CC genotype (Pro/Pro). These differences can be attributed to the sample size and to ethnic differences across the studied subjects (Stefanaki *et al.*, 2007). We found no evidence for an effect of the *TP53* codon 72 SNP in relation to clinicopathological characteristics, age of onset, or to recurrence. Our results support previous findings (Han *et al.*, 2006; Povey *et al.*, 2007; Firoz *et al.*, 2009; Jiang *et al.*, 2011).

MDM2, the other key factor of the TP53 pathway, is overexpressed in many cancers; and mouse models that overexpress *MDM2* are more susceptible to cancer development (Jones *et al.*, 1998). In humans, overexpression of *MDM2* has been described in the absence of genetic amplification in invasive and metastatic melanomas (Polsky *et al.*, 2001), and in association with disease-free survival (relative risk: 0.47; 95% CI: 0.24–0.89) and overall survival (relative risk: 0.55; 95% CI: 0.33–0.94) (Polsky *et al.*, 2002). These findings were confirmed in a large study of 405 melanomas in which the

Table 3. Effect of *MDM2* and *TP53* genotypes on overall mortality, disease-specific mortality, and recurrence

	Overall mortality			Disease-specific mortality			Recurrence		
	HR[1]	95% CI	*P*-value	HR[1]	95% CI	*P*-value	HR[1]	95% CI	*P*-value
MDM2 SNP309			0.197			0.188			0.902
TT	1.00			1.00			1.00		
TG	0.79	(0.56–1.12)		0.74	(0.47–1.15)		1.07	(0.80–1.43)	
GG	0.68	(0.43–1.06)		0.59	(0.32–1.10)		1.04	(0.73–1.50)	
TP53 c.72			0.290			0.131			0.421
GG	1.00			1.00			1.00		
GC	0.76	(0.54–1.07)		0.62	(0.39–0.99)		0.83	(0.63–1.09)	
CC	0.88	(0.49–1.60)		0.81	(0.38–1.74)		0.98	(0.61–1.59)	

Abbreviations: CI, confidence interval; HR, hazard ratio; MDM2, human homolog of mouse double minute 2; SNP, single-nucleotide polymorphism; TP53, tumor protein p53.
[1]Adjusted for age, gender, thickness, stage at diagnosis, and anatomic site.

authors found higher levels of nuclear *MDM2* expression in primary than in metastatic melanomas (Berger *et al.*, 2004). The precise beneficial effect of MDM2 is still not well understood, but may involve a p53-independent cell cycle arrest and inhibition of cell proliferation (Brown *et al.*, 1998; Deb, 2003; Berger *et al.*, 2004).

Several polymorphisms have been identified within *MDM2*, but the SNP309 T>G SNP at a regulatory site in intron 1 has been the most studied. In the presence of the G allele, the coactivator of the estrogen receptor specific protein 1 transcription factor binds to *MDM2* more efficiently than when the T allele is present (Bond *et al.*, 2004). This led to the hypothesis that individuals carrying the G allele would exhibit higher levels of MDM2, especially in the presence of estrogens, with the subsequent reduction of tumor-suppressing function of p53 (Bond *et al.*, 2006a). In our study, we did not find statistically significant associations between the *MDM2* SNP309 T>G polymorphism and clinicopathological variables. However, when we stratified the groups into gender and decades of age at diagnosis, we found an increased frequency of the GG genotype among women diagnosed at an older age, which does not support the hypothesis of an active estrogen signaling via the SNP309 GG genotype. Another study conducted in 249 cases and 291 controls found that women having the SNP309 GG genotype were diagnosed at an older age, although the association did not reach statistical significance (Capasso *et al.*, 2010). The same authors found a positive association between the GG genotype and tumor thickness (Capasso *et al.*, 2010). Nan *et al.* (2009) conducted a nested case–control study of the Nurses' Health Study among 219 cases and found no associations between SNP309 and risk or age of onset. They also tested 3,207 women pooled from controls of three nested case–control studies within the Nurses' Health Study and found that the G allele was inversely associated with the number of moles on the arm (adjusted OR: 0.68; 95% CI: 0.53–0.87 for GG vs. TT) and an increased tendency for childhood tanning (adjusted OR: 1.30; 95% CI: 1.01–1.68).

SNP309 G allele has been proposed to mediate the increase in MDM2 mRNA and protein levels. In our study, we found a non-statistically reduced OR in relation to melanoma death. We cannot speculate on the basis of our results that increased levels of MDM2 might be associated with better survival. However, interestingly and as mentioned earlier, overexpression of *MDM2* in melanomas in the absence of genetic amplification has indeed been reported in association with better survival (Polsky *et al.*, 2001).

Our results do not support the findings from Firoz *et al.* (2009), who reported that among the 89 genotyped women, carriers of the GG genotype were nearly 4-fold more likely to be diagnosed at an early age compared with women with the T allele (unadjusted OR: 3.89; 95% CI: 1.22–12.31). The difference in our findings is likely because of the much larger sample size ($n=990$, including 421 women and 569 men), which allowed us to adjust the analysis for potential confounders. In addition, our cohort included patients who were younger, had thicker tumors, and were at higher stages of the disease.

Collectively, the studies by Nan *et al.* (2009), Capasso *et al.* (2010), and ours agree in that the *MDM2* SNP309 G does not appear to be a risk factor for developing melanoma at a young age. A very recent report unveils a second *MDM2* promoter SNP in position 285 (SNP285G>C), which forms a distinct haplotype with SNP309 (SNP285C/SNP309G) (Knappskog *et al.*, 2011). Although the G allele of SNP309 enhances the transcription, the C allele of SNP285 has a much stronger effect and reduces the binding of specific protein 1 transcription factor on the promoter. Moreover, SNP285C reduces the risk of both ovarian and breast cancer (Knappskog *et al.*, 2011). Consequently, although we cannot rule out the influence of other modifying genes and/or environmental factors that might be affecting the role of MDM2 in melanoma, it will be important to consider a more

Table 4. Distribution of *MDM2* SNP309 genotypes according to clinicopathological and phenotypic features

	All (*N*=986)				Women (*N*=418)				Men (*N*=568)			
	TT (*N*=360)	TG (*N*=451)	GG (*N*=175)	*P*-value[1]	TT (*N*=147)	TG (*N*=194)	GG (*N*=77)	*P*-value[1]	TT (*N*=213)	TG (*N*=257)	GG (*N*=98)	*P*-value[1]
Age at diagnosis (years), mean (SD)	53.5 (15.6)	54.1 (15.0)	55.9 (16.6)	0.16	**48.3 (15.8)**	**51.7 (16.5)**	**54.3 (17.7)**	**0.03**	57.1 (14.4)	56.0 (13.6)	57.2 (15.7)	0.47
Tumor thickness (mm), median (IQR)	1.3 (0.7–2.5)	1.5 (0.9–2.7)	1.4 (0.8–2.5)	0.24	1.2 (0.7–2.1)	1.4 (0.7–2.2)	1.2 (0.7–2.1)	0.90	1.4 (0.7–2.6)	1.6 (0.9–3.4)	1.5 (0.8–2.8)	0.10
Stage at diagnosis				0.10				0.68				0.23
0	21 (30.4)	37 (53.6)	11 (15.9)		12 (35.3)	17 (50.0)	5 (14.7)		9 (25.7)	20 (57.1)	6 (17.1)	
I	190 (38.9)	203 (41.5)	96 (19.6)		80 (35.6)	98 (43.6)	47 (20.9)		110 (41.7)	105 (39.8)	49 (18.6)	
II	77 (35.3)	102 (46.8)	39 (17.9)		31 (34.8)	41 (45.1)	17 (19.1)		46 (35.7)	61 (47.3)	22 (17.1)	
⩾III	66 (33.2)	108 (54.3)	28 (14.1)		22 (33.3)	36 (54.6)	8 (12.1)		44 (37.1)	69 (51.9)	20 (15.0)	
Anatomic site				0.10				0.65				0.15
Head/face	23 (31.9)	37 (51.4)	12 (16.7)		6 (33.3)	11 (61.1)	1 (5.6)		17 (31.5)	26 (48.2)	11 (20.4)	
Trunk	138 (41.1)	136 (40.5)	62 (18.5)		39 (39.4)	41 (41.4)	19 (19.2)		99 (41.8)	95 (40.1)	43 (18.2)	
Extremity	185 (34.5)	253 (47.2)	98 (18.3)		96 (33.7)	134 (47.0)	55 (19.3)		89 (33.5)	119 (47.4)	43 (17.1)	
Unknown	14 (33.3)	25 (59.5)	3 (7.1)		6 (37.5)	8 (50.0)	2 (12.5)		8 (30.8)	17 (65.3)	1 (3.9)	
Family history of melanoma	67 (38.5)	77 (44.3)	30 (17.2)	0.80	29 (34.9)	35 (42.2)	19 (22.9)	0.49	38 (41.8)	42 (46.2)	11 (12.1)	0.35
Number of moles				0.20				0.57				0.11
None	89 (34.5)	121 (46.9)	48 (18.6)		39 (37.5)	46 (44.2)	19 (18.2)		50 (32.5)	75 (48.7)	29 (18.8)	
Few	190 (38.6)	215 (43.7)	87 (17.7)		76 (36.0)	93 (44.1)	42 (19.9)		114 (40.6)	122 (43.4)	45 (16.0)	
Moderate	63 (38.2)	74 (44.8)	28 (17.0)		21 (29.6)	38 (53.5)	12 (16.9)		42 (44.7)	36 (38.3)	16 (17.0)	
Many	7 (17.5)	24 (60.0)	9 (22.5)		3 (20.0)	10 (66.7)	2 (13.3)		4 (16.0)	14 (56.0)	7 (28.0)	
Phenotypic index				0.60				0.32				0.56
1	37 (34.6)	49 (45.8)	21 (19.6)		17 (35.4)	17 (35.4)	14 (29.2)		20 (33.9)	32 (54.2)	7 (11.9)	
2	66 (34.2)	90 (46.6)	37 (19.2)		26 (32.9)	49 (50.6)	13 (16.5)		40 (35.1)	50 (43.9)	24 (21.1)	
3	134 (36.6)	166 (45.4)	66 (18.0)		47 (34.1)	63 (45.6)	28 (20.3)		87 (38.2)	103 (45.2)	38 (16.7)	
4	86 (39.3)	98 (44.7)	35 (16.0)		34 (34.4)	48 (48.5)	17 (17.2)		52 (43.3)	50 (41.7)	18 (15.0)	
5	29 (37.2)	42 (53.8)	7 (9.0)		18 (40.9)	23 (52.3)	3 (6.8)		11 (32.4)	19 (55.6)	4 (11.8)	

Abbreviations: IQR, interquartile range; MDM2, human homolog of mouse double minute 2; SD, standard deviation; SNP, single-nucleotide polymorphism; TP53, tumor protein p53.
Note: numbers within parentheses represent row percent, except for genotype, age at diagnosis, and tumor thickness variables. Significant results appear in bold type.
[1] *P*-values were calculated with the χ^2 test.

complex interaction with the haplotypes SNP285C/SNP309G in future investigations. Considering the fact that the SNP285 appears to be a young SNP present among Western Europeans but absent in Asians, it is interesting to note that two meta-analyses found that the effect of SNP309 on cancer risk is race specific, with SNP309 exerting its effect in Asians but not in Caucasians, where SNP285 can oppose its function (Gui *et al.*, 2009; Economopoulos and Sergentanis, 2010). Future studies are needed to determine whether SNP285C is responsible for the delayed onset among women with melanoma who carry SNP309 G alleles.

One limitation of this study is that we were not able to statistically correct for population stratification because of a lack of ancestry-informative markers in this cohort. However, we conducted a separate analysis on self-identified non-Hispanic whites, which has been shown to sufficiently reduce population stratification bias in well-designed cancer cohort studies (Wacholder *et al.*, 2002). This is especially true for case-only melanoma studies, where the non-white population is a very small minority. Importantly, the results obtained from this hospital cohort may not necessarily be generalized for all melanomas. We believe, however, that

there is a plausible biological explanation for our findings, and that there are several strengths of our study, including its prospective cohort design, which includes extensive patient characteristics and follow-up of patient outcomes. Moreover, to the best of our knowledge, this study comprises the largest number of melanoma cases tested for these SNPs to date. The findings reported here need to be replicated in an independent and larger study to rule out competing influences of chance and population stratification and to investigate the influence of the surrounding genetic environment.

In summary, in this study we did not find an indication for melanoma recurrence, survival, or an earlier age of onset of melanoma in carriers of either *MDM2* SNP309 or *TP53* codon 72 Arg/Pro variant alleles. Our results suggest that women carrying the SNP309 GG genotype might be at a lower risk of developing melanoma at a young age compared with those carrying TG or TT. It is possible to hypothesize that our observations are the result of the antagonistic effect of the nearby functional SNP; however, this remains to be tested and corroborated in future studies. It is premature to attribute clinical and public health usefulness of our findings at this time; however, identifying host factors that modify age of onset or outcomes could be informative in individuals at risk.

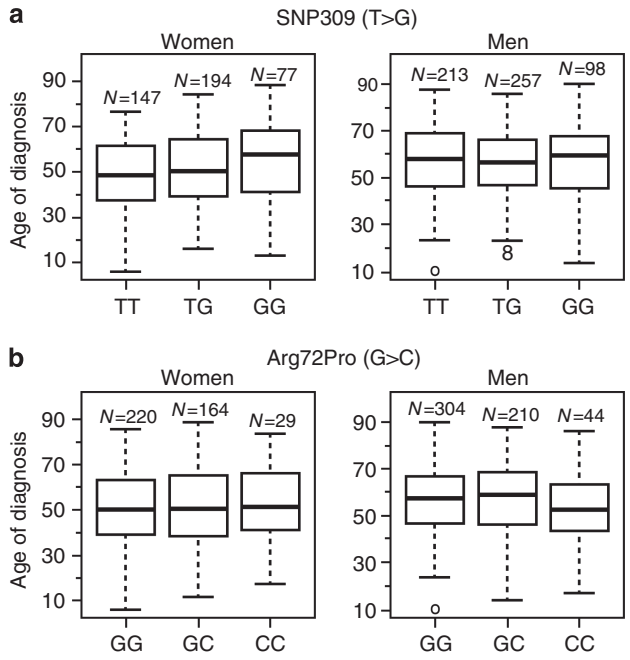

Figure 1. Age at diagnosis in melanoma patients according to *MDM2* SNP309 or *TP53* Arg72Pro genotype and gender. The box plots display the age distribution of melanoma diagnosis by gender for (**a**) *MDM2* SNP309 and (**b**) *TP53* Arg72Pro. The boxes represent the interquartile range; the horizontal thick line within each of the boxes represents the median age of diagnosis. The dotted lines extend from the 1st quartile to the smallest non-outlier value and from the 3rd quartile to the maximum non-outlier. Outliers are indicated by open circles. The number of melanoma patients is indicated for each genotype. Note the higher median age at diagnosis among the women carriers of the SNP309 G allele. Arg, arginine; MDM2, human homolog of mouse double minute 2; Pro, proline; SNP, single-nucleotide polymorphism; TP53, tumor protein p53.

MATERIALS AND METHODS

Study population

A total of 990 patients with cutaneous malignant melanoma (stages 0–IV) were recruited between February 2001 and August 2005 at Memorial Sloan-Kettering Cancer Center (MSKCC), New York. The study protocol was approved by the Memorial Sloan-Kettering Cancer Center Institutional Review Board, and the study was conducted according to the Declaration of Helsinki Principles. In all, 96% of the eligible individuals agreed to participate in the study. All participants signed an informed consent and research authorization. Patients filled out a short, self-administered questionnaire that included information on gender, race, age, family history, density of freckles, hair color, eye color, propensity to burn, and ability to tan after sun exposure. The information on hair and eye color, and propensity to tan or sunburn were combined into a single variable, the phenotypic index (Millikan *et al.*, 2006). This phenotypic index represents the sum of points assigned to hair color (brown/black: 1; light brown/blond: 2; red/auburn: 3); eye color (brown: 0; green/hazel/blue: 1); and propensity to tan (0) or to sunburn (1). We also obtained information on dysplastic nevi, multiple primary melanomas, stage at diagnosis and at follow-up (based on the American Joint

Table 5. ORs for age of onset of melanoma for carriers of the *MDM2* SNP309 GG genotype

Age category	*N*	OR (95% CI)[1]	*P*-value	OR_{adj} (95% CI)[1,2]	*P*-value	OR_{adj} (95% CI)[1,3]	*P*-value
Women (total N=*418)*							
<50 vs. ⩾50	207 vs. 211	0.60 (0.35–0.98)	0.04	0.60 (0.35–1.00)	0.05	0.52 (0.29–0.92)	0.02
<60 vs. ⩾60	282 vs. 136	0.51 (0.31–0.84)	<0.01	0.47 (0.29–0.84)	<0.01	0.42 (0.24–0.75)	<0.01
Men (total N=*568)*							
<50 vs. ⩾50	184 vs. 384	0.90 (0.56–1.45)	0.67	0.97 (0.59–1.60)	0.91	0.90 (0.53–1.53)	0.71
<60 vs. ⩾60	319 vs. 249	0.81 (0.52–1.26)	0.36	0.91 (0.57–1.43)	0.67	0.91 (0.55–1.48)	0.69

Abbreviations: CI, confidence interval; MDM2, human homolog of mouse double minute 2; OR, odds ratio; SNP, single-nucleotide polymorphism.
[1]Crude ORs were calculated for carriers of GG compared to those with GT or TT.
[2]OR_{adj}, OR adjusted for stage at diagnosis, Breslow thickness, and family history.
[3]OR_{adj}, OR adjusted for stage at diagnosis, Breslow thickness, family history, phenotypic index, and nevi.

Committee on Cancer 2002 classification), disease status, disease recurrence, and survival. The characteristics of the study group are shown in Table 1.

Biospecimens

Germline DNA was extracted from buccal cells ($N = 990$) using Puregene kits (Gentra Systems, Minneapolis, MN) following the manufacturer's recommendations. DNA concentration and quality were measured at 260 and 280 nm in a SpectramaxPlus 384 (Molecular Devices, Sunnyvale, CA).

Genotyping

We genotyped samples for the *MDM2* SNP309 (rs2279744; g.31345886 T>G) and the *TP53* Arg72Pro (rs1042522; g.7176821G>C) polymorphisms using PCR-based methods. For the *MDM2* SNP309, genotyping was carried out by pyrosequencing (Ronaghi, 2003) with the PSQTM HS96A instrument (Biotage AB, Uppsala, Sweden). The PCR reactions included 10–15 ng DNA, 200 μM dNTP (Invitrogen, Carlsbad, CA), 2 mM $MgCl_2$ (Applied Biosystems, Foster City, CA), 0.025 U μl^{-1} of *Taq* polymerase (Applied Biosystems), 1 × reaction buffer, and 1 μM of each primer (forward: 5′-GGGCTGCGGGGCC GCT-3′; reverse: 5′-biotin-AGTGACCCGACAGGCACCT-3′) (Invitrogen). Cycling included a step at 95°C for 5 minutes, followed by 45 cycles of 95°C for 20 seconds, 55°C for 20 seconds, 72°C for 20 seconds, with a final step at 72°C for 5 minutes. The MDM2 sequencing primer (5′-GGGCTGCGGGGCCGCT-3′) (Invitrogen) was added to the single-stranded DNA and nucleotides were dispensed automatically.

For the *TP53* polymorphism, genotyping was carried out by differential melting temperature analysis (Bennett *et al.*, 2003) coupled to the LightTyper Instrument (Roche Applied Science, Indianapolis, IN). Briefly, the PCR reactions contained 10–15 ng DNA, 200 μM dNTP (Invitrogen), 3.25 mM $MgCl_2$ (Applied Biosystems), 0.025 U μl^{-1} of *Taq* polymerase (Applied Biosystems), 1 × reaction buffer, 0.04 μM forward primer: 5′-ATGGATGATTTGATGCTGT-3′, 0.6 μM reverse primer: 5′-CAGAATGCAAGAAGCCC-3′ (Invitrogen), and 0.15 μM of labeled probes (anchor, 5′-AGATGAAGCTCCCAGAATGCCAG-fluorescein; sensor, 5′-LCRed640-GCTGCTCCCCCCGTG-phosphate) (Roche Applied Science). The cycling consisted of a denaturation at 95°C for 5 minutes, followed by 50 cycles at 95°C for 30 seconds, 55°C for 20 seconds, 72°C for 30 seconds, with a final extension at 72°C for 5 minutes.

All genotyping included blanks and known internal positive controls. Samples that failed to amplify were repeated. Samples with results that departed from the expected profiles for wild type, heterozygote, or variant were sequenced to confirm specificity. To this effect, DNA was amplified in an independent PCR, purified, and then 10 ng of the product sequenced in an ABI 3730-XLDNA Analyzer (Applied Biosystems). Additional quality control assurance consisted of 5% randomly repeated samples. Assays ''passed'' in the absence of signal in the negative (blank) controls, in the presence of the expected signal or profile for the known internal controls, and when there was 100% agreement between two independent readers, and between blinded repeats added for quality control. Genotyping was successful in 99.6 and 98.1% of the samples for the *MDM2* SNP309 (rs2279744) and the *TP53* Arg72Pro (rs1042522) SNPs, respectively. The genotypes for both SNPs were in Hardy–Weinberg equilibrium.

Statistical analysis

χ^2 Tests and Wilcoxon rank-sum test were used to test for associations between the following: (i) clinicopathological and phenotypic characteristics of the patients and gender; (ii) genotypes and various clinical and epidemiological factors by gender, and between the *MDM2* and *TP53* genotypes. Multivariable logistic regression, adjusting for thickness, stage, family history, phenotypic index, and nevi, was conducted to examine the effect of the *MDM2* SNP309 GG genotype on age of melanoma diagnosis.

Overall survival and disease-specific survival were calculated from the date of initial diagnosis of melanoma to date of death or last follow-up. Time to recurrence was calculated from the date of initial diagnosis to date of first recurrence. Overall survival, disease-specific survival, and time to recurrence were estimated using Kaplan–Meier methods. The effect of genotype on survival outcomes was evaluated with the Cox proportional model after adjusting for clinicopathological factors, including age at diagnosis, stage, thickness, gender, and site, and were selected *a priori* based on published reports (de Vries *et al.*, 2008; Lachiewicz *et al.*, 2008; Balch *et al.*, 2009). We corrected *P*-values for multiple hypothesis testing using the Benjamini–Hochberg method (Benjamini and Hochberg, 1995). All statistical tests were two sided and were carried out using SAS version 9.1 (SAS Institute, Cary, NC).

CONFLICT OF INTEREST

The authors state no conflict of interest.

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SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at http://www.nature.com/jid

REFERENCES

Alhopuro P, Ylisaukko-Oja SK, Koskinen WJ *et al.* (2005) The MDM2 promoter polymorphism SNP309T→G and the risk of uterine leiomyosarcoma, colorectal cancer, and squamous cell carcinoma of the head and neck. *J Med Genet* 42:694–8

Balch CM, Gershenwald JE, Soong SJ *et al.* (2009) Final version of 2009 AJCC melanoma staging and classification. *J Clin Oncol: Off J Am Soc Clin Oncol* 27:6199–206

Benjamini Y, Hochberg Y (1995) Controlling the false discovery rate: a practical and powerful approach to multiple testing. *J R Stat Soc Ser B* 57:289–300

Bennett CD, Campbell MN, Cook CJ *et al.* (2003) The LightTyper: high-throughput genotyping using fluorescent melting curve analysis. *Biotechniques* 34:1288–92, 1294–1295

Berger AJ, Camp RL, Divito KA *et al.* (2004) Automated quantitative analysis of HDM2 expression in malignant melanoma shows association with early-stage disease and improved outcome. *Cancer Res* 64:8767–72

Bond GL, Hirshfield KM, Kirchhoff T *et al.* (2006a) MDM2 SNP309 accelerates tumor formation in a gender-specific and hormone-dependent manner. *Cancer Res* 66:5104–10

Bond GL, Hu W, Bond EE *et al.* (2004) A single nucleotide polymorphism in the MDM2 promoter attenuates the p53 tumor suppressor pathway and accelerates tumor formation in humans. *Cell* 119:591–602

Bond GL, Menin C, Bertorelle R *et al.* (2006b) MDM2 SNP309 accelerates colorectal tumour formation in women. *J Med Genet* 43:950–2

Box NF, Terzian T (2008) The role of p53 in pigmentation, tanning and melanoma. *Pigment Cell Melanoma Res* 21:525–33

Brown DR, Thomas CA, Deb SP (1998) The human oncoprotein MDM2 arrests the cell cycle: elimination of its cell-cycle–inhibitory function induces tumorigenesis. *EMBO J* 17:2513–25

Capasso M, Ayala F, Avvisati RA *et al.* (2010) MDM2 SNP309 and p53 Arg72Pro in cutaneous melanoma: association between SNP309 GG genotype and tumor Breslow thickness. *J Hum Genet* 55:518–24

de Vries E, Nijsten TE, Visser O *et al.* (2008) Superior survival of females among 10,538 Dutch melanoma patients is independent of Breslow thickness, histologic type and tumor site. *Ann Oncol* 19:583–9

Deb SP (2003) Cell cycle regulatory functions of the human oncoprotein MDM2. *Mol Cancer Res* 1:1009–16

Economopoulos KP, Sergentanis TN (2010) Differential effects of MDM2 SNP309 polymorphism on breast cancer risk along with race: a meta-analysis. *Breast Cancer Res Treat* 120:211–6

Ellis NA, Huo D, Yildiz O *et al.* (2008) *MDM2* SNP309 and *TP53* Arg72Pro interact to alter therapy-related acute myeloid leukemia susceptibility. *Blood* 112:741–9

Fang S, Krahe R, Lozano G *et al.* (2010) Effects of MDM2, MDM4 and TP53 codon 72 polymorphisms on cancer risk in a cohort study of carriers of TP53 germline mutations. *PLoS One* 5:e10813

Firoz EF, Warycha M, Zakrzewski J *et al.* (2009) Association of MDM2 SNP309, age of onset, and gender in cutaneous melanoma. *Clin Cancer Res* 15:2573–80

Gluck I, Simon AJ, Catane R *et al.* (2009) Germline analysis of thymidine/guanidine polymorphism at position 309 of the Mdm2 promoter in malignant melanoma patients. *Melanoma Res* 19:199–202

Gui XH, Qiu LX, Zhang HF *et al.* (2009) MDM2 309 T/G polymorphism is associated with lung cancer risk among Asians. *Eur J Cancer* 45: 2023–2026

Gwosdz C, Scheckenbach K, Lieven O *et al.* (2006) Comprehensive analysis of the p53 status in mucosal and cutaneous melanomas. *Int J Cancer* 118:577–82

Han J, Cox DG, Colditz GA *et al.* (2006) The p53 codon 72 polymorphism, sunburns, and risk of skin cancer in US Caucasian women. *Mol Carcinogen* 45:694–700

Horner M, Ries L, Krapcho M *et al.* (2009) *SEER Cancer Statistics Review, 1975–2006.* National Cancer Institute: Bethesda, Maryland

Jemal A, Siegel R, Xu J *et al.* (2010) Cancer statistics, 2010. *CA Cancer J Clin* 60:277–300

Jiang DK, Wang WZ, Ren WH *et al.* (2011) TP53 Arg72Pro polymorphism and skin cancer risk: a meta-analysis. *J Invest Dermatol* 131:220–8

Jones SN, Hancock AR, Vogel H *et al.* (1998) Overexpression of Mdm2 in mice reveals a p53-independent role for Mdm2 in tumorigenesis. *Proc Natl Acad Sci USA* 95:15608–12

Joosse A, de Vries E, Eckel R *et al.* (2011) Gender differences in melanoma survival: female patients have a decreased risk of metastasis. *J Invest Dermatol* 131:719–26

Kinyamu HK, Archer TK (2003) Estrogen receptor-dependent proteasomal degradation of the glucocorticoid receptor is coupled to an increase in mdm2 protein expression. *Mol Cell Biol* 23:5867–81

Knappskog S, Bjornslett M, Myklebust LM *et al.* (2011) The MDM2 promoter SNP285C/309G haplotype diminishes Sp1 transcription factor binding and reduces risk for breast and ovarian cancer in Caucasians. *Cancer Cell* 19:273–82

Krekac D, Brozkova K, Knoflickova D *et al.* (2008) MDM2SNP309 does not associate with elevated MDM2 protein expression or breast cancer risk. *Oncology* 74:84–7

Lachiewicz AM, Berwick M, Wiggins CL *et al.* (2008) Survival differences between patients with scalp or neck melanoma and those with melanoma of other sites in the Surveillance, Epidemiology, and End Results (SEER) program. *Arch Dermatol* 144:515–21

Lasithiotakis K, Leiter U, Meier F *et al.* (2008) Age and gender are significant independent predictors of survival in primary cutaneous melanoma. *Cancer* 112:1795–804

Li C, Chen K, Liu Z *et al.* (2008) Polymorphisms of TP53 Arg72Pro, but not p73 G4C14>A4TA4 and p21 Ser31Arg, contribute to risk of cutaneous melanoma. *J Invest Dermatol* 128:1585–8

Marin MC, Jost CA, Brooks LA *et al.* (2000) A common polymorphism acts as an intragenic modifier of mutant p53 behaviour. *Nat Genet* 25:47–54

Matlashewski GJ, Tuck S, Pim D *et al.* (1987) Primary structure polymorphism at amino acid residue 72 of human p53. *Mol Cell Biol* 7:961–3

Millikan RC, Hummer A, Begg C *et al.* (2006) Polymorphisms in nucleotide excision repair genes and risk of multiple primary melanoma: the Genes Environment and Melanoma Study. *Carcinogenesis* 27:610–8

Nan H, Qureshi AA, Hunter DJ *et al.* (2009) A functional SNP in the MDM2 promoter, pigmentary phenotypes, and risk of skin cancer. *Cancer Causes Control* 20:171–9

Okumura N, Saji S, Eguchi H *et al.* (2002) Distinct promoter usage of mdm2 gene in human breast cancer. *Oncol Rep* 9:557–63

Polsky D, Bastian BC, Hazan C *et al.* (2001) HDM2 protein overexpression, but not gene amplification, is related to tumorigenesis of cutaneous melanoma. *Cancer Res* 61:7642–6

Polsky D, Melzer K, Hazan C *et al.* (2002) HDM2 protein overexpression and prognosis in primary malignant melanoma. *J Natl Cancer Inst* 94:1803–6

Povey JE, Darakhshan F, Robertson K *et al.* (2007) DNA repair gene polymorphisms and genetic predisposition to cutaneous melanoma. *Carcinogenesis* 28:1087–93

Ronaghi M (2003) Pyrosequencing for SNP genotyping. *Methods Mol Biol* 212:189–95

Schmidt MK, Reincke S, Broeks A *et al.* (2007) Do MDM2 SNP309 and TP53 R72P interact in breast cancer susceptibility? A large pooled series from the breast cancer association consortium. *Cancer Res* 67:9584–90

Shen H, Liu Z, Strom SS *et al.* (2003) P53 codon 72 Arg homozygotes are associated with an increased risk of cutaneous melanoma. *J Invest Dermatol* 121:1510–4

Stefanaki I, Stratigos AJ, Dimisianos G *et al.* (2007) P53 codon 72 Pro homozygosity increases the risk of cutaneous melanoma in individuals with dark skin complexion and among noncarriers of melanocortin 1 receptor red hair variants. *Br J Dermatol* 156:357–62

Thomas M, Kalita A, Labrecque S *et al.* (1999) Two polymorphic variants of wild-type p53 differ biochemically and biologically. *Mol Cell Biol* 19:1092–100

Wacholder S, Rothman N, Caporaso N (2002) Counterpoint: bias from population stratification is not a major threat to the validity of conclusions from epidemiological studies of common polymorphisms and cancer. *Cancer Epidemiol Biomarkers Prev* 11:513–20

Wilkening S, Bermejo JL, Hemminki K (2007) MDM2 SNP309 and cancer risk: a combined analysis. *Carcinogenesis* 28:2262–7

Yu H, Huang YJ, Liu Z *et al.* (2011) Effects of MDM2 promoter polymorphisms and p53 codon 72 polymorphism on risk and age at onset of squamous cell carcinoma of the head and neck. *Mol Carcinogen* 50:697–706