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Association between genetic polymorphisms in apoptosis-related genes and risk of cutaneous melanoma in women and men

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ABSTRACT

Background: The *P53* Arg72Pro, *MDM2* c.+309T > G, *BAX* c.–248G > A, and *BCL2* c.–717C > A polymorphisms have variable roles in the apoptosis pathways.
Objective: To clarify the roles of these polymorphisms in the risk for cutaneous melanoma (CM).
Methods: Genomic DNA of 200 CM patients and 215 controls was analyzed by PCR–RFLP.
Results: In women, the frequencies of *BAX* GG (83.0% vs. 71.0%, $P = 0.04$), *BCL2* AA (32.0% vs. 15.0%, $P = 0.003$), *P53* ArgArg plus *BAX* GG (84.9% vs. 63.2%, $P = 0.01$), *P53* ArgArg plus *BCL2* AA (37.0% vs. 13.1%, $P = 0.003$), *BAX* GG plus *BCL2* AA (70.3% vs. 33.3%, $P = 0.001$), *MDM2* GG plus *BAX* GG plus *BCL2* AA (27.3% vs. 3.7%, $P = 0.03$), and *P53* ArgArg plus *MDM2* GG plus *BAX* GG plus *BCL2* AA (33.3% vs. 5.6%, $P = 0.04$) genotypes were higher in patients than in controls. Female carriers of the respective genotypes were under 1.98 (95% CI: 1.01–3.91), 2.87 (95% CI: 1.43–5.77), 3.48 (95% CI: 1.34–9.04), 4.23 (95% CI: 1.63–10.96), 6.04 (95% CI: 2.10–17.37), 25.61 (95% CI: 1.29–507.24), and 25.69 (95% CI: 1.11–593.59)-fold increased risks for CM than others, respectively. In men, the frequencies of *BCL2* CA + AA (83.0% vs. 67.6%, $P = 0.01$) and *MDM2* TG + GG plus *BCL2* CA + AA (94.2% vs. 68.3%, $P = 0.003$) genotypes were higher in patients than in controls. Male carriers of the respective genotypes were under 2.43 (95% CI: 1.23–4.82) and 9.22 (95% CI: 2.16–39.31)-fold increased CM risks than others, respectively.
Conclusion: The data suggest for the first time that *P53* Arg72Pro, *MDM2* c.+309T > G, *BAX* c.–248G > A, and *BCL2* c.–717C > A polymorphisms, enrolled in apoptosis pathways, constitute distinct determinants of CM in women and men.

1. Introduction

Exposure to ultraviolet (UV) radiation from the sunlight, including UVA and UVB components, is considered the most important environmental risk factor for developing cutaneous melanoma (CM) [1].

UVA and UVB damage repair in epithelial cell DNA is required to maintain genome integrity and apoptosis failures may initiate the photo-carcinogenic process and originate CM [2,3]. The p53 protein promotes DNA repair and apoptosis [4]. The Mdm2 protein binds directly to and inhibits p53, regulating its location, stability, and activity as a transcriptional activator [5,6]. The Bax protein promotes cell death via apoptosis [7], whereas its homologous protein, Bcl2, inhibits cell death [8], under regulation of the p53 transcriptional factor [9].

It is already well established that abilities to induce apoptosis are variable in humans [10,11]. A *P53* single nucleotide polymorphism (SNP) is located at the 72nd amino acid residue, with an arginine (Arg) to proline (Pro) change because of a G→C transversion (Arg72Pro, rs1042522) [12], the protein encoded by Arg allele is more efficient in inducing apoptosis than that encoded by Pro allele [13]. A SNP located in promoter region of *MDM2* gene is characterized by a T→G substitution at the +309 nucleotide position (c.+309T > G, rs2279744) [14]. The protein encoded by G allele increases the affinity of the transcriptional activator specificity protein 1 (Sp1) for the *MDM2* promoter, resulting in higher expression of Mdm2 when compared with T allele, and subsequent attenuation of p53 pathway [14]. The *BAX* SNP with a G→A substitution at –248 nucleotide position (c.–248G > A, rs4645878) is located in the 5′-untranslated region [15]. The G

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allele was associated with higher protein levels [16], and also with lower transcriptional activity [17] when compared with A allele. The *BCL2* SNP with a C→A substitution at –717 nucleotide position (c.–717C > A, rs2279115) is located on the *BCL2* gene promoter [18] and AA genotype was associated with increased Bcl2 expression in comparison with CC genotype [19,20].

The roles of *P53* Arg72Pro and *MDM2* c.+309T > G SNPs, analyzed predominantly in Caucasians from Europe and North America and Asians, in CM risk are controversial [21–30]. In addition, to the best of our knowledge, the roles of *BAX* c.–248G > A and *BCL2* c.–717C > A SNPs in CM risk are still unknown.

Conversely, melanin also protects skin from UV radiation, and is regulated by estrogen and androgen [31], which contributes to CM pathogenesis [32–34]. Sex hormones also alter *P53*, *MDM2*, *BAX*, and *BCL2* expression [35–39].

The Brazilian population is heterogeneous, mixed, and composed of Amerindians and European, Asian and African immigrants [40]. Furthermore, Brazilians have been highly exposed to UV rays, and the incidence of CM is rising rapidly in the country [41]. Since analyzes of various distinct populations are necessary to define the roles of genetic polymorphisms in the origin of a certain disease, the identification of *P53* Arg72Pro, *MDM2* c.+309T > G, *BAX* c.–248G > A and *BCL2* c.–717C > A SNPs in women and men highly exposed to UV rays was considered necessary to test their influences on CM risk.

2. Materials and methods

2.1. Study population

The case group comprised 200 consecutive CM patients at diagnosis (median age: 55 years, range: 20–89; 100 women, 100 men) followed at the Clinical Oncology and Dermatology Services of the University Hospital from June 2007 to March 2013. The control group comprised 215 healthy blood donors matched by gender and skin color (median age: 52 years, range: 23–60; 107 women and 108 men) followed at the same University Hospital during the same period of time in order to provide a representative group of the general population that seeks medical assistance in our hospital. Individuals with a personal or family history of CM and those who did not accept to participate of the study were excluded from the analyses. All procedures were carried out according to the Declaration of Helsinki.

Information obtained from a standardized questionnaire included self-reported host characteristics. Patients were classified according to light or non-light skin color, light (blue/green) or dark (brown/black) eye color and light (red/blond) or dark (brown/black) natural hair color. The numbers of nevi over the entire skin surface of patients were classified as none (0), few (1–20), moderate (21–50), and many (>50) [42]. Freckles were classified as none/few (limited to a single body part) and moderate/many (more than two body areas) [43]. The classification of skin phototypes (I to VI) was performed in accordance with the Fitzpatrick Classification Scale [44], considering constitutional skin color and the result of UV radiation exposure (I, white, very fair, red or blond hair, blue eyes, freckles, always burns and never tans; II, white, fair, red or blond hair, blue, hazel or green eyes, usually burns and tans with difficulty; III, white, fair with any eye or hair color, sometimes mild burn and gradually tans; IV, brown, typical Mediterranean Caucasian skin, rarely burns, tans with ease; V, dark brown, middle-eastern skin types, very rarely burns and tans very easily; VI, black, never burns and tans very easily). Sunburn episodes were defined as pain and erythema and/or blisters for more than 24 h in childhood [45]. Sun exposure was classified as intermittent or chronic [46]. Sun exposure time of patients was classified in less than, equal to, or greater than 20 years [45]. The tumor site was classified into axial (head, neck and trunk) and peripheral (upper and lower limbs), and the diagnosis of CM was histologically confirmed. The invasion depth and tumor stage were identified using Breslow (millimeters) and Clark (I–V) levels [47] and the American Joint Committee on Cancer "Melanoma Staging System" criteria [48], respectively.

2.2. Genetic polymorphism analysis

Genotyping was performed in genomic DNA of subjects' peripheral blood samples using polymerase chain reaction followed by enzymatic digestion, as previously reported for *P53* Arg72Pro (rs1042522) [49], *MDM2* c.+309T > G (rs2279744) [50], *BAX* c.–248G > A (rs4645878) [51], and *BCL2* c.–717C > A (rs2279115) [20] polymorphisms. The amount of 10–15% of genotype determinations was carried out twice in independent experiments with 100% of concordance.

2.3. Statistical analysis

The Hardy–Weinberg (HW) equilibrium was tested using the Chi-square (χ^2) statistics for the goodness-of-fit test. The differences between groups were analyzed by the χ^2 or Fisher's exact test. Multivariate analysis was performed using the logistic regression model and served to obtain age and skin color adjusted crude odds ratios (ORs) and assess the associations among genotypes and CM. Power of analysis (PA) was used to calculate the minimum effect size that is likely to be detected in a study using a given sample size. PA was calculated in analyses involving patients and controls, according to Pocock (1983) [52] and Hulley et al. (1988) [53], and using DSS Research Statistical Power Calculators (http://www.dssresearch.com/Knowledge Center/toolkitcalculators/statisticalpowercalculators.aspx) in analyses of groups of patients stratified by clinical aspects and tumor characteristics. Statistical significance was established at $P<0.05$ and all tests were done using the SPSS 15.0 software (SPSS Incorporation, Chicago, IL, USA). To evaluate genetic interaction among the polymorphisms and gender in our sample, we used the multifactor dimensionality reduction (MDR) model, which is a nonparametric and genetic model-free data mining for nonlinear interaction identification among genetic and environmental attributes [54–56]. To adjust results for multiple comparisons, we performed a MDR permutation test in our sample, totalizing 100,000 permutations. The MDR test was performed using MDR 2.0 and MDRPT 0.4.7 software.

3. Results

Similar clinical characteristics of patients and tumor biological aspects were seen in female and male patients. Only tumor location and distribution differed in patients stratified by gender: females presented tumor predominately in upper/lower limbs while tumors in head, neck, and trunk were more common in males (Table 1).

Samples of controls (women and men) were in HW equilibrium at all analyzed loci. Female patient samples were in HW equilibrium at *P53* Arg72Pro, *MDM2* c.+309T > G, and *BCL2* c.–717C > A loci but not at *BAX* c.–248G > A locus. Male patient samples confirmed HW expectations at *MDM2* c.+309T > G, *BAX* c.–248G > A, and *BCL2* c.–717C > A loci, but not at *P53* Arg72Pro locus (Table 2).

3.1. Association between genotypes and cutaneous melanoma risk

The frequencies of the genotypes and alleles of the *P53* Arg72Pro, *MDM2* c.+309T > G, *BCL2* c.–717C > A, and *BAX* c.–

Table 1
Clinical characteristics of cutaneous melanoma patients stratified by gender.

Clinical characteristics	*N*=100	Women (%)	*N*=100	Men (%)	*P* value
Median age (years)		52		56	0.27
Range		20–89		25–88	
Skin color					
Light	100	91 (91.0)	100	92 (92.0)	1.00
Non-light		9 (9.0)		8 (8.0)	
Eye color					
Light (blue/green)	86*	22 (25.6)	85*	24 (28.2)	0.73
Dark (brown/black)		64 (74.4)		61 (71.8)	
Hair color					
Light (blond/red)	86*	20 (23.3)	85*	25 (29.4)	0.38
Dark (brown/black)		66 (76.7)		60 (70.6)	
Common nevi					
None/few (0–20)	94*	35 (37.2)	97*	48 (49.5)	0.10
Moderate (21–50)		40 (42.5)		39 (40.2)	
Many (>50)		19 (20.3)		10 (10.3)	
Freckles					
None/few	93*	43 (46.2)	95*	42 (44.2)	0.24
Moderate/many		50 (53.8)		53 (55.8)	
Phototype					
I–II	84*	43 (51.2)	82*	38 (46.3)	0.53
III–V		41 (48.8)		44 (53.7)	
Sunburns in childhood					
Yes	96*	55 (57.3)	80*	59 (73.7)	0.18
No		41 (42.7)		21 (26.3)	
Type of sun exposure					
Intermittent	46*	13 (28.3)	56*	17 (30.4)	0.83
Chronic		33 (71.7)		39 (69.6)	
Sun exposure					
≤ 20 years	73*	48 (65.7)	75*	44 (58.7)	0.40
> 20 years		25 (34.3)		31 (41.3)	
Tumor site					
Head and neck/trunk	95*	43 (45.3)	96*	61 (63.5)	**0.006**
Upper/lower limb		52 (54.7)		35 (36.5)	
Breslow					
<0.76 mm	95*	37 (39.0)	86*	27 (31.4)	0.35
≥ 0.76 mm		58 (61.0)		59 (68.6)	
Clark					
I + II	97*	37 (38.1)	89*	25 (28.1)	0.16
III + IV + V		60 (61.9)		64 (71.9)	
AJCC stage					
I + II	88*	69 (78.4)	91*	62 (68.1)	0.13
III + IV		19 (21.6)		29 (31.9)	

* The total numbers of individuals differed from the total quoted (n = 100), because it was not possible to obtain consistent information about characteristics in some individuals; *P* values<0.05 are presented in bold letters; AJCC, American Joint Committee on Cancer.

248G > A polymorphisms in patients and controls stratified by gender are presented in Table 3.

In the women group, *BAX* GG (83.0% vs. 71.0%, *P*= 0.04, PA: 52%) and *BCL2* AA (32.0% vs. 15.0%, *P*= 0.003, PA: 90%)-isolated genotypes were more common in patients than in controls; female carriers of the respective genotypes were under 1.98 (95% CI: 1.01–3.91) and 2.87 (95% CI: 1.43–5.77)-fold increased CM risks than those with the remaining genotypes, respectively. Excesses of *P53* ArgArg plus *BAX* GG (84.9% vs. 63.2%, *P*= 0.01, PA: 96%), *P53* ArgArg plus *BCL2* AA (37.0% vs. 13.1%, *P*= 0.003, PA: 99%), *BAX* GG plus *BCL2* AA (70.3% vs. 33.3%, *P*= 0.001, PA= 99%), *MDM2* GG plus *BAX* GG plus *BCL2* AA (27.3% vs. 3.7%, *P*= 0.03, PA: 99%), and *P53* ArgArg plus *MDM2* GG plus *BAX* GG plus *BCL2* AA (33.3% vs. 5.6%, *P*= 0.04, PA: 99%) combined genotypes were seen in patients when compared with controls. Female carriers of the respective genotypes were under 3.48 (95% CI: 1.34–9.04), 4.23 (95% CI: 1.63–10.96), 6.04 (95% CI: 2.10–17.37), 25.61 (95% CI: 1.29–507.24), and 25.69 (95% CI: 1.11–593.59)-fold increased CM risks than those with the remaining genotypes, respectively (Table 3).

In the men group, *BCL2* CA + AA (83.0% vs. 67.6%, *P*= 0.01, PA: 75%) and *MDM2* TG + GG plus *BCL2* CA + AA (94.2% vs. 68.3%, *P*= 0.003, PA: 99%) genotypes were more common in patients than in controls. Male carriers of the respective genotypes were under 2.43 (95% CI: 1.23–4.82) and 9.22 (95% CI: 2.16–39.31)-fold increased CM risks than those with the remaining genotypes (Table 3).

Table 2
Hardy–Weinberg equilibrium analyses of single nucleotide polymorphisms identified in patients with cutaneous melanoma and controls stratified by gender.

Gender	SNP	Patients		Controls	
		χ^2	*P* value	χ^2	*P* value
Women	*P53* Arg72Pro	0.62	0.42	0.13	0.71
	MDM2 c.+309T > G	0.08	0.77	0.01	0.92
	BAX c.–248G > A	**4.80**	**0.02**	3.32	0.06
	BCL2 c.–717C > A	0.05	0.82	0.04	0.84
Men	*P53* Arg72Pro	**6.53**	**0.001**	0.08	0.77
	MDM2 c.+309T > G	0.77	0.37	0.08	0.77
	BAX c.–248G > A	0.01	0.92	1.95	0.27
	BCL2 c.–717C > A	0.18	0.66	2.87	0.09

SNP, single nucleotide polymorphism. Loci in Hardy–Weinberg disequilibrium are presented in bold letters.

3.2. Association between genotypes and clinical and tumor characteristics

Only the significant associations between genotypes and clinical and tumor characteristics in female and males patients with CM are presented in Tables 4 and 5, respectively.

Table 3
Frequencies of *P53* Arg72Pro, *MDM2* c.+309T > G, *BAX* c.–248G > A, and *BCL2* c.–717C > A genotypes and alleles in cutaneous melanomas and controls stratified by gender.

Polymorphisms	Women					Men				
	Patients (%) *n*=100	Controls (%) *n*=107	*P* value	OR* (95% CI)	PA	Patients (%) *n*=100	Controls (%) *n*=108	*P* value	OR* (95% CI)	PA
P53 Arg72Pro										
ArgArg	54 (54.0)	46 (43.0)	0.13	1.52 (0.87–2.68)	31%	59 (59.0)	50 (46.3)	0.15	1.51 (0.86–2.66)	31%
ArgPro + ProPro	46 (46.0)	61 (57.0)		reference		41 (41.0)	58 (53.7)		reference	
ArgArg + ArgPro	95 (95.0)	93 (86.9)	0.08	2.56 (0.86–7.57)	45%	88 (88.0)	96 (88.9)	0.74	0.86 (0.35–2.09)	1%
ProPro	5 (5.0)	14 (13.1)		reference		12 (12.0)	12 (11.1)		reference	
Arg allele	75.0	65.0	0.16			73.0	67.0	0.44		
Pro allele	25.0	35.0				27.0	33.0			
MDM2 c.+309T > G										
TT	39 (39.0)	41 (38.3)	0.68	reference	9%	37 (37.0)	50 (46.3)	0.37	reference	15%
TG + GG	61 (61.0)	66 (61.7)		0.84 (0.37–1.90)		63 (63.0)	58 (53.7)		1.29 (0.72–2.31)	
TT + TG	87 (87.0)	91 (85.0)	0.95	reference	<1%	88 (88.0)	96 (88.9)	0.86	reference	<1%
GG	13 (13.0)	16 (15.0)		0.98 (0.55–1.73)		12 (12.0)	12 (11.1)		0.92 (0.38–2.22)	
T allele	63.0	61.0	0.88			62.0	67.0	0.55		
G allele	37.0	39.0				38.0	33.0			
BAX c.–248G > A										
GG	83 (83.0)	76 (71.0)	**0.04**	1.98 (1.01–3.91)	52%	80 (80.0)	91 (84.2)	0.36	1.40 (0.67–2.91)	13%
GA + AA	17 (17.0)	31 (29.0)		reference		20 (20.0)	17 (15.8)		reference	
GG + GA	99 (99.0)	104 (97.2)	0.41	2.61 (0.26–25.71)	14%	99 (99.0)	106 (98.1)	0.82	1.31 (0.11–15.15)	<1%
AA	1 (1.00)	3 (2.8)		reference		1 (1.0)	2 (1.9)		reference	
G allele	91.0	84.0	0.19			89.0	91.0	0.81		
A allele	0.09	16.0				10.0	0.09			
BCL2 c.–717C > A										
CC	29 (29.0)	29 (27.1)	0.63	reference	8%	17 (17.0)	35 (32.4)	0.01	reference	75%
CA + AA	71 (71.0)	78 (72.9)		0.86 (0.6–1.59)		83 (83.0)	73 (67.6)		2.43 (1.23–4.82)	
CC + CA	68 (68.0)	91(85.0)	**0.003**	reference	90%	68 (68.0)	80 (74.0)	0.37	reference	14%
AA	32 (32.0)	16 (15.0)		2.87 (1.43–5.77)		32 (32.0)	28 (26.0)		1.32 (0.71–2.44)	
C allele	48.0	52.0	0.67			42.0	53.0	0.15		
A allele	52.0	48.0				58.0	47.0			
P53 + *BAX*										
ArgArg + GG	45 (84.9)	36 (63.2)	**0.01**	3.48 (1.34–9.04)	96%	45 (88.3)	44 (80.0)	0.44	1.55 (0.50–4.80)	26%
ArgPro + ProPro + GA + AA	8 (15.1)	21 (36.8)		reference		6 (11.7)	11 (20.0)		reference	
P53 + *BCL2*										
ArgArg + AA	20 (37.0)	8 (13.1)	**0.003**	4.23 (1.63–10.96)	99%	18 (40.0)	15 (25.0)	0.12	1.93 (0.83–4.50)	60%
ArgPro + ProPro + CC + CA	34 (63.0)	53 (86.9)		reference		27 (60.0)	45 (75.0)		reference	
MDM2 + *BCL2*										
TT + CC	10 (19.2)	10 (17.3)	0.71	reference	8%	3 (5.8)	20 (31.7)	0.003	reference	99%
TG + GG + CA + AA	42 (80.8)	48 (82.7)		0.83 (0.30–2.27)		49 (94.2)	43 (68.3)		9.22 (2.16–39.31)	
BAX + *BCL2*										
GG + AA	26 (70.3)	15 (33.3)	**0.001**	6.04 (2.10–17.37)	99%	26 (65.0)	19 (70.4)	0.53	1.41 (0.47–4.17)	5%
GA + AA + CC + CA	11 (29.7)	30 (66.7)		reference		14 (35.0)	8 (29.6)		reference	
MDM2 + *BAX* + *BCL2*										
TT + TG + GA + AA + CC + CA	8 (72.7)	26 (96.3)	**0.03**	reference	99%	60 (92.3)	71 (97.3)	0.13	reference	50%
GG + GG + AA	3 (27.3)	1 (3.7)		25.61 (1.29–507.24)		5 (7.7)	2 (2.7)		3.63 (0.66–19.97)	
P53 + *MDM2* + *BAX* + *BCL2*										
ArgPro + ProPro + TT + TG + GA + AA + CC + CA	6 (66.7)	17 (94.4)	**0.04**	reference	99%	5 (55.5)	8 (88.9)	0.11	reference	99%
ArgArg + GG + GG + AA	3 (33.3)	1 (5.6)		25.69 (1.11–593.59)		4 (45.5)	1 (11.1)		19.80 (0.48–17.16)	

OR*, odds ratio adjusted by age and skin color by the multivariate analysis; CI, confidence interval; PA, power of analysis; *P* values < 0.05 are presented in bold letters.

Table 4
Associations between genotypes and clinical and tumor characteristics in females with cutaneous melanoma.

SNP/clinical characteristics	Genotypes		Statistical results	
P53 Arg72Pro	ArgArg (%)	ArgPro + ProPro (%)	*P* value	PA
Sun exposure				
Intermittent	6 (46.2)	7 (53.8)	0.03	27%
Chronic	21 (63.6)	12 (36.4)		
MDM2	TT (%)	TG + GG (%)		
Phototype				
I–II	21 (48.8)	22 (51.2)	0.008	64%
III–V	10 (24.4)	31 (75.6)		
Eye color				
Light (green/blue)	13 (59.1)	9 (40.9)	0.01	69%
Dark (brown/black)	19 (29.7)	45 (70.3)		
Hair color				
Light (blond/red)	13 (65.0)	7 (35.0)	0.003	84%
Dark (brown/black)	19 (28.8)	47 (71.2)		

SNP, single nucleotide polymorphism; *P* values are presented herein after adjustment by age and skin color by the multivariate analysis; PA, power of analysis.

P53 ArgArg genotype was more common in female patients with chronic sun exposure than in those with intermittent sun exposure (63.6% vs. 46.2%, *P*= 0.03, PA: 27%). Genotype frequency was also higher in the first group of patients than in female controls (63.6% vs. 43.0%, *P*= 0.04, PA: 40%). Women with *P53* ArgArg genotype who were chronically exposed to sun had a 2.32 (95% CI: 1.03–5.19)-fold CM increased risk than others. *MDM2* TG + GG genotype was more common in female patients with skin phototypes III–VI than in those with phototypes I + II (75.6% vs. 51.2%, *P*=0.008, PA: 64%), in female patients with dark eyes than in those with light eyes (70.3% vs. 40.9%, *P*= 0.01, PA: 69%), and in female patients with dark hair than in those with light hair (71.2% vs. 35.0%, *P*= 0.003, PA: 84%) (Table 4).

Excesses of *P53* ArgArg + ArgPro (90.2% vs. 62.5%, *P*= 0.02, PA: 59%), *P53* ArgArg + ArgPro plus *MDM2* TG + GG (96.3% vs. 60.0%, *P*= 0.01, PA: 73%) genotypes were seen in male patients with light skin color when compared with those with non-light skin. *MDM2* GG plus *BCL2* AA (28.6% vs. 5.1%, *P*= 0.04, PA: 56%) genotypes were

Table 5
Associations between genotypes and clinical and tumor characteristics in males with cutaneous melanoma.

SNP/clinical characteristics	Genotypes		Statistical results	
P53 Arg72Pro	ArgArg + ArgPro (%)	ProPro (%)	*P* value	PA
Skin color				
Light	83 (90.2)	9 (9.8)	0.02	59%
Non-light	5 (62.5)	3 (37.5)		
P53 + MDM2	ArgPro + ArgArg + TG + GG (%)	ProPro + TT (%)		
Skin color				
Light	52 (96.3)	2 (3.7)	0.01	73%
Non-light	3 (60.0)	2 (40.0)		
MDM2 + BCL2	TT + TG + CC + CA (%)	GG + AA (%)		
Skin color				
Light	56 (94.9)	3 (5.1)	0.04	56%
Non-light	5 (71.4)	2 (28.6)		
BCL2	CC + CA (%)	AA (%)		
Eye color				
Light	11 (45.8)	13 (54.2)	0.01	62%
Dark	44 (72.1)	17 (27.9)		
Hair color				
Light (blond/red)	11 (44.0)	14 (56.0)	0.01	72%
Dark (brown/black)	44 (73.3)	16 (26.7)		

SNP, single nucleotide polymorphism; *P* values are presented herein after adjustment by age and skin color by the multivariate analysis; PA, power of analysis.

more common in male patients with non-light skin color when compared with patients with light skin. The frequency of *BCL2* AA genotype was higher in male patients with light eyes and light hair than in those with dark eyes and dark hair (54.2% vs. 27.9%, *P*= 0.01, PA: 62%; 56.0% vs. 26.7%, *P*= 0.01, PA: 72%; respectively), and also than in male controls (54.2% vs. 25.9%, *P*= 0.01, PA: 50%; 56.0% vs. 25.9%, *P*= 0.007, PA: 57%; respectively). Men with *BCL2* AA genotype with light eyes and light hair had 3.37 (95% CI: 1.35–8.39) and 3.63 (95% CI: 1.47–8.94)-fold increased CM risks than carriers of the remaining genotypes (Table 5).

The MDR results indicated that there was positive interaction between combined genotypes of *P53*, *MDM2*, *BAX*, and *BCL2* and gender in our population (*P*= 0.0408). No association between other polymorphisms, isolated or combined, and gender was found in study (data not shown).

4. Discussion

In the late 1960s, Clark et al. (1969) [57] observed that CM was more aggressive in men. Since then, numerous studies suggested differences in tumor–host interaction across gender [33]. Based on these, we investigated herein whether *P53* Arg72Pro, *MDM2* c.+309T > G, *BAX* c.–248G > A, and *BCL2* c.–717C > A polymorphisms alter risk, demographic characteristics, and biological features of tumor in Brazilian CM patients stratified by gender.

The distributions of our cases by tumor clinical and biological aspects were, in general, similar to those found in other countries [21,25], indicating that our sample was representative of the disease in the world. No consistent differences were seen in female and male patients of group, and therefore, individuals could be analyzed separately in a study focusing on inherited genetic abnormalities in CM origin.

We observed that *P53* Arg72Pro SNP did not alter CM risk in women or men, in accordance with an Italian study [27]. In both studies, subjects were analyzed after stratification by gender. In addition, a previous study conducted by us [30] showed an excess of *P53* ArgArg genotype in CM patients when compared with controls, when we analyzed the total subjects, suggesting that stratification by gender may alter the role of *P53* Arg72Pro in the disease. *P53* Arg72Pro was associated with increased CM risk [21,23,25,28] or did not alter CM risk [22,24,29] in previous reports considering women and men together. In fact, *P53* is activated upon UV damage and initiates a transcriptional program that leads to DNA repair or apoptosis, [4] and abnormalities in these functions conferred by Pro [13] and Arg [58] alleles of *P53* Arg72Pro in apoptosis and DNA repair, respectively, may lead skin cells to cancer. However, our data suggest that *P53* Arg72Pro SNP alone may have no effect on tumor risk in Brazilian individuals stratified by gender.

We found no association of *MDM2* c.+309T > G SNP and CM risk in women and men, as previously reported by Capasso et al. (2010) [27] and Nan et al. (2009) [26], also found no association of SNP with CM risk, considering all subjects pooled. It is already known that Mdm2 protein binds directly to p53, resulting in subsequent degradation of the protein [14]. *MDM2* c.+309G allele increases the DNA-binding affinity of the transcriptional activator Sp1 to the gene promoter and the expression of mRNA and protein compared to T allele, resulting in higher attenuation of p53 pathway [14]. Estrogen also binds to promoter region and activates MDM2 transcription [36], and *MDM2* c.+309T > G SNP accelerates the formation of various tumors in a gender-specific and hormone-dependent manner [37,38], even that no influence of *MDM2* c.+309T > G SNP with CM had been seen in subjects stratified by gender in our study.

BAX GG genotype was associated with increased CM risk in women in our study. To the best of our knowledge, there are no previous studies focusing on the influence of *BAX* c.–248G > A SNP on CM risk. In fact, Bax protein plays a role in releasing apoptosis-related factors [7]. However, the role of *BAX* c.–248G > A SNP in gene expression is controversial: G allele was associated with higher mRNA and protein levels [16] and exhibited lower transcriptional activity [17] when compared with A allele in different studies. Our data suggest a role for G allele in reducing apoptosis of skin cells. Our data also suggest that CM development might differ according to sex, since we could observe the influence of *BAX* c.–248G > A SNP only in women. In fact, it seems that a biological trait that differs according to sex affects melanoma in a profound way [33]. Accordingly, biological sex differences in estrogen [59] and androgen [60] receptor expressions could be involved in cell proliferation and pathogenesis of CM. Recently, Simões et al. (2013) [39] showed that estradiol and testosterone could act as apoptotic modulators, down-regulating *P53* and *BAX* mRNA levels and preventing apoptosis by activation of mitogen-activated protein kinase pathways.

Higher frequencies of *BCL2* AA and CA + AA genotypes were seen in female and male CM patients of our sample, respectively. To the best of our knowledge, there are no studies focusing on the influence of *BCL2* c.–717C > A SNP on CM risk. In fact, *BCL2* gene plays a key role in protecting cancer cells from apoptosis [8] and AA genotype of *BCL2* c.–717C > A SNP was associated with increased Bcl2 expression when compared with CC genotype [19,20]. Therefore, our data suggest that *BCL2* c.717C > A SNP alters CM risk in women and men in our country.

Associations of *P53* ArgArg plus *BAX* GG, *P53* ArgArg plus *BCL2* AA, *BAX* GG plus *BCL2* AA, *MDM2* GG plus *BAX* GG plus *BCL2* AA, and *P53* ArgArg plus *MDM2* GG plus *BAX* GG plus *BCL2* AA genotypes with increased risk of CM in women were seen in our study, suggesting a synergic action of the four related apoptosis genes in CM development in women. In fact, the pathway of suppressor tumor p53-dependent apoptosis involves Mdm2, an inhibitor of p53, and pro-apoptotic protein Bax and anti-apoptotic protein Bcl2 [4,61]. In addition, proteins encoded by G allele of *MDM2* c.+309T > G [14] and *BAX* c.–248G > A [17], as well as A allele

of *BCL2* c.–717C > A [19,20]. SNPs were previously described as more efficient to maintain cell survival than those encoded by the remaining alleles and may have acted together in CM development in our cases. Arg protein of *P53* Arg72Pro SNP was seen as less efficient in DNA repair than Pro protein [61] and may have contributed to CM onset in our cases.

MDM2 TG + GG plus *BCL2* CA + AA combined genotype was associated with increased risk of CM only in men in our study, suggesting a synergic action of *MDM2* and *BCL2* genes in CM [14,19,20]. Again, exposure of subjects to sex hormones could make difference in *P53* [39,62], *MDM2* [36,63], *BAX* [39,64], and BCL2 [35] expressions in women and men involved in the study. As far as our knowledge reaches, no studies focusing on analyses of combined SNPs in CM risk were previously conduced.

We found an association of *P53* ArgArg genotype with increased CM risk in women chronically exposed to sunlight, possible due to the action of Arg protein in accumulating DNA damage of UV light in skin cells [61]. We found that *MDM2* TG + GG genotypes were more frequent in female patients with skin phototypes III–VI and dark eyes and hair when compared with others. The protein encoded by G allele results in a high Mdm2 expression [14], contributing to cell survival, but its association with phenotypic characteristics considered as protective factors against CM was unexpected [43]. In addition, *P53* ArgArg + ArgPro isolated or combined with *MDM2* TG + GG genotype was more common in male patients with light skin color than in others, and *MDM2* GG plus *BCL2* AA genotype was more common in male patients with non-light skin color than in others in our study. *BCL2* AA genotype was also associated with increased CM risk in men with light eyes and light hair. In fact, p53 pathway may be deregulated during melanomagenesis [65], and proteins encoded by G allele of *MDM2* c.+309T > G[14] and A allele of *BCL2* c.–717C > A [19,20] contributed to the maintenance of cell survival. No previous studies were conducted focusing the association of SNPs with clinical and CM aspects. Again, distinct exposures of individuals to sex hormones may explain the differences in associations seen in women and men analyzed herein [35,36,39,59,62–65].

Finally, we would like to highlight genome-wide association studies (GWAS) that found SNPs located in genes with apoptotic functions that were related to CM risk. Amos et al. (2011) observed that the region of chromosome 1q21.3 near the *LASS2* gene, a ceramide synthase, was highly associated with CM risk [66]. In addition, in another GWAS, Barrett et al. (2011) observed that several SNPs adjacent to *CASP8* gene were associated with melanoma susceptibility [67].

In conclusion, for the first time, our data present evidences that *P53* Arg72Pro, *MDM2* c.+309T > G, *BAX* c.–248G > A, and *BCL2* c.–717C > A SNPs alter the risk and clinical aspects of CM in a population from southeastern Brazil stratified by gender. We recognize that our conclusions are based on relatively small numbers of individuals and require confirmation by additional larger studies. If the associations between SNPs and increased CM risk in our tropical country are confirmed, we might be able to identify a high-risk subset of the population, who could benefit from a more rigorous control of sun exposure and skin surveillance.

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