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POMC and *TP53* genetic variability and risk of basal cell carcinoma of skin: Interaction between host and genetic factors

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ABSTRACT

Background: Basal cell carcinoma (BCC) of the skin is the most common neoplasm among the Caucasian population of the western world. Ultraviolet (UV) radiation-induced p53 activation promotes cutaneous pigmentation by increasing transcriptional activity of pro-opiomelanocortin (POMC) in the skin. Induction of POMC/α-melanocyte-stimulating hormone (α-MSH) activates the melanocortin 1 receptor (MC1R), resulting in skin pigmentation. The tumor suppressor p53 is a key player in stress responses that preserve genomic stability, responding to a variety of insults including DNA damage, hypoxia, metabolic stress and oncogene activation. Malfunction of the p53 pathway is an almost universal hallmark of human tumors. Polymorphisms in the gene encoding p53 (*TP53*) alter its transcriptional activity, which in turn may influence the UV radiation-induced tanning response.
Objective: The aim of the present work is to test association between *POMC* and *TP53* genetic variability, the possible interplay with host factors and the risk of basal cell carcinoma of skin.
Methods: We covered the variability of the two genes we used 17 tagging polymorphisms in 529 BCC cases and 532 healthy controls. We have also tested the possible interactions between the genetic variants and three known risk factors for BCC: skin complexion, sun effect and skin response to sun exposure.
Results: We did not observe any statistically significant association between SNPs in these two genes and BCC risk overall, nor interactions of SNPs with known BCC risk factors. However we found that, in the group of subjects with lower sun exposure, carriers of one copy of the C allele of the *TP53* SNP rs12951053 had a decreased risk of BCC (OR = 0.28, 95% CI 0.12–0.62, $P = 0.002$).
Conclusions: We have observed that the interplay of an environmental risk factor and one polymorphism in *TP53* gene could modulate the risk of BCC.

1. Introduction

Basal cell carcinoma BCC of the skin is the most common neoplasm among the Caucasian population of the western world [1]. The risk for development of BCC is mainly associated with environmental factors (especially sun exposure) but also with genetic factors [2].

One of the main risk factors for BCC is ultraviolet (UV) radiation, through its induction of DNA damage [3–5]. Tanning is acquired pigmentation that results from exposure to UV, melanin synthesis by cutaneous melanocytes and transport into adjacent keratinocytes [6].

Melanin production is initiated by α-melanocyte-stimulating hormone (α-MSH), which is produced by proteolysis from a multicomponent precursor polypeptide, encoded by the pro-opiomelanocortin (POMC) gene [7]. The induced POMC/α-MSH binds to MC1R, which further activates the cyclic adenosine monophosphate (cAMP) signalling system, leading to eumelanin production.

It has been shown that p53 is involved in cell-cycle arrest and apoptosis in response to UV-induced DNA damage [8,9]; moreover,

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Cui et al. [10] showed that the tumor-suppressor protein p53 promotes cutaneous pigmentation following UV irradiation by direct transcriptional activation of POMC in the skin and that p53 absence ablates the tanning response.

TP53 mutations have been detected in about half of all BCCs. Furthermore, it was found that aggressive forms of BCC are significantly associated with increased p53 expression [1,11].

This tumor suppressor gene is highly polymorphic: so far over 200 SNPs have been identified http://p53.free.fr; http://www-p53.iarc.fr/ [12]. Few of the many *TP53* polymorphisms have been assessed for altered biochemical and/or biological function, or for their effects on cancer risk in population studies [13]. The association between p53 codon 72 polymorphism and BCC risk has been investigated but the findings are controversial [14–17]. Another study investigated the association between BCC cancer risk and one SNP in the *POMC* gene with negative results [18].

In this study we investigated the effect of tagging and functional polymorphisms in the entire *TP53* and *POMC* genes on well characterized BCC cases and controls. We analyzed TP53 and POMC jointly with *MC1R* variants, which had been previously genotyped in the same cases and controls [19].

2. Materials and methods

2.1. Study population

A set of newly diagnosed cases and controls were recruited as part of a large study on risk of various cancers due to environmental arsenic exposure in Hungary, Romania and Slovakia between 2002 and 2004 [20]. The recruitment was carried out in the counties of Bacs, Csongrad and Jasz-Nagykun-Szolnok in Hungary; Bihor and Arad in Romania and Nitra in Slovakia. Skin cancer cases (n = 529) were invited on the basis of histopathological examinations by pathologists. Hospital-based controls (n = 532) were included in the study, subject to fulfillment of a set of criteria. All general hospitals in the study area were involved in the process of control recruitment and a rotation scheme was used in order to achieve appropriate geographical distribution. The controls were surgery, orthopedic and trauma patients with conditions such as appendicitis, abdominal hernias, duodenal ulcers, cholelithiasis and fractures; patients with malignant tumors, diabetes and cardiovascular diseases were excluded. The matching of the controls was done using this criteria: being of the same gender of the index case belonging to the same 5 years age band (30–34, 35–39, etc.), being of the same area. Moreover cases and controls were recruited among those individuals that have resided in the study area for at least one year during their lifetime) [21].

Subsequent to the signing of consent forms by the participants, clinicians took venous blood from cases and controls. The blood samples were kept deep frozen at −80 °C until analysis. A general questionnaire was completed by trained personnel after an interview of the recruited cases and controls. The questionnaire was designed to include information on individual cumulative sun exposure in summer, sun-tanning, skin-complexion, effects of sun-exposure on skin and age/s at diagnosis of BCC; the Fitzpatrick classification was not used because of non-availability of facilities uniformly across all recruiting centers of the participating countries. In addition, the interviews included items on demographic, life-style, socio-economic, medical history, occupational exposures, drinking and nutritional habits, as well as detailed residential history. Ethnic background for the cases and controls was recorded along with other characteristics of the study population. Local ethical boards approved the study plan and design.

2.2. Selection of polymorphisms

We aimed at surveying the entire set of common genetic variants in *TP53* and *POMC* genes. To this end we followed a hybrid functional/tagging approach using the algorithm described by Carlson and co-workers [22]. All polymorphisms in the region of *TP53* gene including 5 kb upstream of the first exon and 5 kb downstream of the last exon with minor allele frequency MAF ≥5%

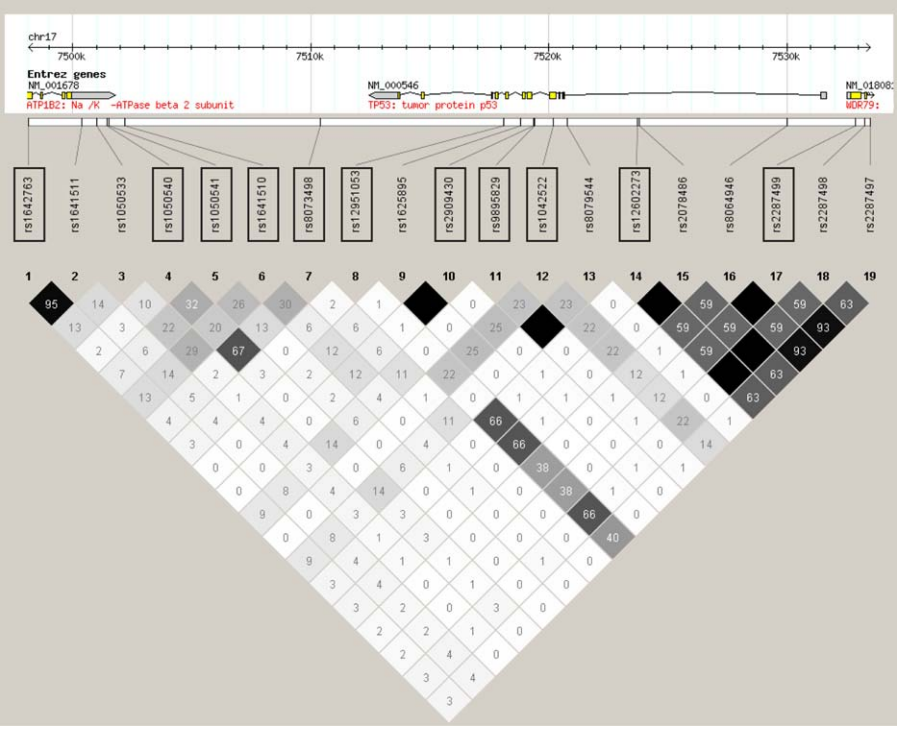

Fig. 1. Linkage disequilibrium (LD) plot across the *TP53* locus, r^2 values are indicated in the plot. Selected SNPs are highlighted in square.

in Caucasians from the International HapMap Project version 26 (http://www.hapmap.org) were included. Tagging SNPs were selected with the use of the Tagger program within Haploview (http://www.broadinstitute.org/mpg/haploview/; http://www.broadinstitute.org/mpg/tagger/) [23,24], using pairwise tagging with a minimum r^2 of 0.8 (Fig. 1). We also included additional tagging SNPs selected among polymorphisms detected in a previous study [12]. We forced in the selection of tagging SNPs also rs1042522 Pro72Arg, which is a non-synonymous variant possibly implicated in risk of several cancer types. The SNP rs2909430 tags two other polymorphisms already found associated with cancer risk, a 16 bp duplication in the intron 3 rs17878362 [25] and a SNP in intron 6 rs1625895 [25].

SNPs selection for *POMC* has been described in a previous work [26] in which the selection has been done using an haplotype-tagging approach according to the method of Stram et al. [27], with criterion $r^2_H = 0.7$.

2.3. Genotyping

The order of DNAs from cases and controls was randomized on PCR plates in order to ensure that an equal number of cases and controls could be analyzed simultaneously. All the genotyping was carried out using the Taqman assay. The MGB Taqman probes and primers were synthesized by Applied Biosystems (Foster City, CA, USA). PCRs were performed according to the manufacturer's instructions. PCR plates were read on an ABI PRISM 7900HT instrument by Applied Biosystems. MC1R gene has been genotyped by sequencing the amplification and sequencing conditions have been described previously [19].

2.4. Statistical analysis

The frequency distribution of genotypes was examined for cases and controls. Hardy–Weinberg equilibrium was tested in cases and in controls separately. The host factors, skin complexion and skin response to sun-exposure were categorized into high (H: light complexion or burns/blisters, respectively), medium (M: medium complexion or mild burns) and low (L: dark complexion or tan/no change) risk groups. Sun exposure was estimated by taking a mean of eight categorical variables measuring average daily exposure to the sun in over the respondents' lifetimes. The calculated mean was, then, divided in four categories corresponding to the hours of sun exposure during summer. The four cut-off points were <2.5 h/2.5–3.5 h/3.6–4.5 h/>4.5 h. More detailed information has been previously reported [19].

We used logistic regression for multivariate analyses to assess the main effects of the genetic polymorphism on BCC risk. The primary end point of the analysis was cancer risk, measured with odds ratio and associated confidence intervals. All estimates were adjusted for age at diagnosis, gender, nationality and risk categories.

Considering the large number of comparison performed, we calculated for each gene the number of effective independent variables, M_{eff}, using the SNP Spectral Decomposition approach [28]. We obtained a gene-wide M_{eff} value for each gene and also a study-wide M_{eff} value, by adding up the gene M_{eff}'s. The study-wide M_{eff} value was 12, therefore we applied a study-wise threshold of significance of $P = 0.0042$ (0.05/12), in order to interpret *P*-values in light of the multiple comparisons.

We analyzed associations of SNPs with BCC risk by grouping cases according to age, skin complexion, sun effect and skin response to sun exposure, we analyzed a model with the main effects for each SNP and the covariate of interest and a model where the SNP was parameterized nested within the covariate categories; we then computed the likelihood ratio test between the two models (heterogeneity test). Age was divided in 4 quartiles (<55 years, 55–63 years, 64–72, >72). The host factor subgroups have been described above.

Logistic regression analyses and likelihood ratio test were done with STATA software (StataCorp, College Station, TX, USA).

The haplotype frequencies in cases and controls were inferred with the SAS/Genetics software module (SAS Institute Inc., Cary, NC, U.S.A.) using the expectation–maximization algorithm to generate maximum likelihood estimates. Samples missing one genotype or more were removed from haplotype analyses.

2.5. Gene–gene and gene–environment interactions

We analyzed all the possible pair-wise interactions between SNPs (gene–gene; G–G) and between SNPs and two host factors (skin complexion and skin response to sun exposure) and one environmental factor (sun exposure) (gene-environment; G–E). Assessment of G–G and G–E interactions was carried out using Multifactor Dimensionality Reduction (MDR). The details of MDR are described elsewhere [29–31]. Briefly, MDR is a data reduction approach that seeks to identify combinations of multilocus genotypes and discrete environmental factors that are associated with either high risk or low risk of disease. MDR defines a single variable that incorporates information from several loci and/or environmental factors. This new variable can be evaluated for its ability to classify and predict outcome risk status using cross-validation and permutation testing. The MDR software is open-source and freely available from http://www.epistasis.org.

2.6. Bioinformatics analysis

Potential binding sites of transcription factors within the sequence encompassing the significantly associated SNP were performed with MatInspector Professional http://genomatix.de/cgi-bin/matinspector_prof/mat_fam.pl [32].

3. Results

In this study 529 cases with BCC and 532 controls were recruited from Hungary, Romania and Slovakia. The mean age at

Table 1
Distribution of BCC cases and controls for different characteristics.

Variable	Cases (%)	Controls (%)	*P*-value[a]
Male	237 (44.8)	274 (51.4)	
Female	292 (55.2)	259 (48.6)	
Mean age (±standard deviation)	64.8 (±10.3)	60.0 (±11.8)	
Median age (25–75% percentile)	67 (58–73)	61 (52–70)	
Nationality			
Hungarian	208 (39)	283 (53)	
Romanian	125 (24)	118 (22)	
Slovak	184 (35)	121 (23)	
Others	12 (2)	10 (2)	
Skin complexion			<0.0001
Light	280 (53)	212 (40)	
Medium	233 (44)	261 (49)	
Dark	16 (3)	59 (11)	
Skin response to sun-exposure			0.04
Blistered/burnt	185 (35)	141 (26)	
Mild burn	169 (32)	159 (30)	
Tanning/no change	175 (33)	232 (44)	
Average cumulative sun exposure (h per day during summer)[b]			0.59
<2.4	129 (24)	137 (26)	
2.5–3.5	151 (29)	153 (29)	
3.6–4.5	135 (26)	111 (21)	
>4.5	112 (21)	125 (23)	

[a] *P*-value is for the effect of factor on BCC risk.
[b] Sun exposure estimated by calculating a mean of eight categorical variables measuring average daily exposure to the sun over the respondents' lifetimes. For two cases and six controls exposure information was not available.

diagnosis of the cases (237 men and 292 women) was 64.8 (±10.3) years (median 67; 25–75% percentile 58–73) and that of controls (274 men and 259 women) was 60.0 (±11.8) years (median 61; 25–75% percentile 52–70). While the complexion and nature of skin response to sun exposure showed association with BCC risk, the average cumulative sun-exposure was not associated with the risk Seventeen SNPs in *TP53* and *POMC* genes were genotyped in each subject of the study and analyzed jointly with genotypes of *MC1R* SNPs we generated in a previous study [15]. Baseline characteristics of cases and controls are reported in Table 1. Genotype success rate for cases and controls was greater than 95%. Blinded duplicate samples (6.2%) included for quality control showed genotype concordance >99%. The genotype frequencies for all SNPs were in accordance with Hardy–Weinberg equilibrium in controls, and any deviation from the expected was not statistically significant (data not shown).The distribution of the genotypes and their odds ratios (ORs) for association with BCC risk are shown in Tables 2 and 3. The genotype frequencies of all SNPs were not found to be significantly different between cases and controls.

3.1. G–G, G–E interactions and subgroup analysis

We have thoroughly analyzed all the possible pair-wise G–G and G–E interactions between the selected SNPs in *TP53*, *POMC* and

Table 2
Associations between tagging SNPs in the TP53 gene region and BCC risk.

Genotypes	Position[a]	Cases ($n=529$)[b]	Controls ($n=532$)[b]	OR[c]	95% CI[c]	*p*-Value	*P* trend
rs1642763	7,498,144 *ATP1B2*, exon 4				0.28		
GG		325	343	1 (ref)			
AG		169	145	1.15	(0.87–1.53)	0.322	
AA		24	25	1.06	(0.58–1.96)	0.845	
AG + AA				1.14	(0.87–1.50)	0.338	
rs1050540	7,501,467 *ATP1B2*, 3′ UTR						
CC		212	224	1 (ref)			0.56
CT		239	224	1.09	(0.83–1.44)	0.542	
TT		67	67	0.96	(0.64–1.45)	0.859	
CT + TT				1.06	(0.82–1.37)	0.662	
rs1050541	7,501,560 *ATP1B2*, 3′ UTR						
GG		137	143	1 (ref)			0.93
GT		251	239	1.16	(0.85–1.59)	0.338	
TT		125	133	1.05	(0.73–1.49)	0.802	
GT + TT				1.12	(0.84–1.50)	0.438	
rs1641510	7,502,221 intergenic						
AA		184	171	1 (ref)			0.46
AG		236	247	0.93	(0.70–1.24)	0.628	
GG		100	104	0.94	(0.66–1.35)	0.742	
AG + GG				0.93	(0.71–1.22)	0.62	
rs8073498	7,510,423 intergenic						
AA		184	198	1 (ref)			0.19
AC		247	248	1.06	(0.80–1.40)	0.682	
CC		88	73	1.20	(0.81–1.76)	0.363	
AC + CC				1.09	(0.84–1.43)	0.512	
rs12951053	7,518,132 *TP53*, intron 7						
AA		440	437	1 (ref)			0.55
AC		79	79	0.97	(0.68–1.39)	0.872	
CC		3	7	0.38	(0.09–1.65)	0.198	
AC + CC				0.92	(0.65–1.31)	0.656	
rs2909430	7,519,370 *TP53*, intron 4						
TT		392	387	1 (ref)			0.96
TC		103	112	1.01	(0.73–1.39)	0.950	
CC		15	10	1.52	(0.64–3.60)	0.340	
TC + CC				1.05	(0.77–1.43)	0.741	
rs9895829	7,519,404 *TP53*, intron 4						
GG		390	388	1 (ref)			0.99
GA		106	117	0.99	(0.72–1.36)	0.960	
AA		13	8	1.65	(0.65–4.18)	0.292	
GA + AA				1.04	(0.76–1.40)	0.819	
rs1042522	7,520,197 *TP53*, exon 3						
CC		292	297	1 (ref)			0.91
CG		186	178	1.09	(0.83–1.44)	0.523	
GG		40	46	0.91	(0.57–1.46)	0.702	
CG + GG				1.06	(0.82–1.37)	0.679	
rs12602273	7,523,738 *TP53*, intron 1						
CC		448	441	1 (ref)			0.52
CG		68	78	0.85	(0.59–1.23)	0.385	
GG		3	2	1.31	(0.19–9.2)	0.789	
CG + GG				0.86	(0.6–1.24)	0.418	
rs2287499	7,532,893 *WRAP53*, exon 1						
CC		406	397	1 (ref)			0.19
CG		107	114	0.93	(0.68–1.27)	0.631	
GG		5	12	0.46	(0.15–1.39)	0.169	
CG + GG				0.88	(0.65–1.2)	0.43	

[a] Position of SNP on chromosome 17, in base pairs referred to UCSC Genome Browser on Human Mar. 2006 Assembly. In parentheses we report the position of the polymorphism with respect to the gene. Some SNPs are located in genes immediately flanking *TP53* at the 3′ and 5′.
[b] Numbers may not add up to 100% of subjects due to genotyping failure.
[c] OR: odds ratio; CI: confidence interval. Adjusted for age, gender, nationality and host factors.

Table 3
Associations between tagging SNPs in the *POMC* gene region and BCC risk.

Genotypes	Position[a]	Cases[b]	Controls[b]	OR	95% CI	*P*-value	*P* trend
rs13002622	25,223,520	Intron, *EFR3B*					
TT		248	236	1 (ref)			0.42
TC		223	237	0.88	(0.67–1.15)	0.363	
CC		45	48	0.95	(0.6–1.52)	0.846	
TC + CC				0.89	(0.69–1.16)	0.395	
rs1866146	25,234,077	3′ UTR, *EFR3B*					
AA		186	194	1 (ref)			0.55
AG		261	269	1.09	(0.82–1.44)	0.551	
GG		70	63	1.17	(0.77–1.77)	0.472	
AG + GG				1.10	(0.85–1.44)	0.469	
rs1042571	25,237,391	3′ UTR, *POMC*					
GG		361	366	1 (ref)			0.56
GA		146	149	0.87	(0.65–1.15)	0.327	
AA		14	8	1.96	(0.78–4.97)	0.154	
GA + AA				0.92	(0.69–1.21)	0.546	
rs7566506	25,272,977	Intergenic					
CC		447	447	1 (ref)			0.47
CA		67	73	0.97	(0.67–1.41)	0.871	
AA		3	5	0.64	(0.14–2.85)	0.560	
CA + AA				0.95	(0.66–1.37)	0.775	
rs10202360	25,274,369	Intergenic					
AA		334	327	1 (ref)			0.59
AC		172	175	1.05	(0.80–1.39)	0.711	
CC		14	17	0.80	(0.38–1.72)	0.574	
AC + CC				1.03	(0.79–1.35)	0.827	
rs28932474	25,383,068	Intergenic					
GG		474	475	1 (ref)			0.58
GC		48	54	0.86	(0.56–1.32)	0.479	
CC		0	0				

[a] Position of SNP on chromosome 2, in base pairs referred to UCSC Genome Browser on Human Mar. 2006 Assembly. In parentheses we report the position of the polymorphism with respect to the gene.
[b] Numbers may not add up to 100% of subjects due to genotyping failure.OR: odds ratio; CI: confidence interval. Adjusted for age, gender, nationality and host factors.

MC1R by use of the MDR approach. Analysis using the MDR method with 10-fold cross validation did not reveal any statistically significant interaction (the best model described had cross validation consistency 9/10, test balance accuracy 0.56, OR 1.61 CI 0.74–3.49, *P* value = 0.23). Analyzing the data in subgroups stratifying for age, gender and the host/environmental factors (skin complexion, sun exposure and skin response to sun exposure), we found that two polymorphic variants of *TP53* had a study-wise (*P*-value <0.0041) significant association with BCC risk. Results of subgroup analysis for rs12951053 and rs8073498 are reported in Table 4. Carriers of one copy of the C allele of rs12951053 had a decreased risk of BCC in the group of subjects with lower sun exposure during the summer (OR = 0.28, 95% CI 0.12–0.62, *P* = 0.002). Moreover, carriers of the C allele of the same polymorphism show an increased risk in a group of subject with age ranged between 55 and 63 (OR = 2.67 95% CI 1.27–5.64, *P* = 0.01) and subjects more prone to sun burns (OR = 1.95, 95% CI 0.99–3.81, *P* = 0.052), even if these associations did not reach the threshold for multiple comparison analysis. When stratifying for sun exposure, the SNP rs12951053 had a heterogeneity test *P* value of 0.0187.

We observed an increased risk of BCC in carriers of one copy of the C allele of SNP rs8073498 in *TP53* in the group of subjects with intermediate-high sun exposure during the summer (OR = 2.83, 95% CI 1.50–5.35, *P* = 0.001), although the heterogeneity test was not significant ($p_{\text{heterogeneity}} = 0.43$). Carriers of at least one copy of C allele show an association in this subgroup (OR = 2.32, 95% CI 1.29–4.16, *P* = 0.005), even if this association did not reach the threshold for multiple comparison analysis. All the other polymorphisms, considering the various subgroups, did not show any statistically significant association. Supplementary Tables 1–4 show the analysis of each polymorphism of this study considering the various subgroups of age and sun-related exposures.

Haplotype analysis did not detect associations with BCC risk (showed in detail in Supplementary Table 5).

Searches for potential binding sites of transcription factors were performed with MatInspector within 100 bp sequence surrounding rs12951053 and rs8073498. For the rs12951053 the predicted binding of transcription factor did not differ between the two alleles.

Twenty-one binding sites for transcription factors were detected in the 100 bp sequence surrounding the A allele of rs8073498. Four of those binding sites were abolished in the presence of the C allele (Fig. 2). Two of the abolished sites were for p53 (matrix similarity 0.94 and 0.98) and 2 for Iroquois homeobox transcription factors (not expressed in the skin).

4. Discussion

The risk for development of BCC is mainly associated with environmental factors (especially sun exposure) but also with genetic factors [2]. In this study we investigated the genetic variability of *TP53* and *POMC* genes using a tagging approach and selecting 17 SNPs. Using this method we covered all the known common genetic variation of these genes, including polymorphisms coming from a recent study [12], but we did not find any significant difference of genotype and haplotypes distribution between cases and controls. Polymorphisms in *TP53* have been found to be associated with cancer risk in a variety of tissues [33–35].

Two studies found no association of the p53 codon 72 polymorphism with BCC risk [14,15], while in two others [16,17] the polymorphism has been found associated.

In particular the same population, in which Han et al. reported an association between the p53 codon 72 polymorphism with tanning response for BCC risk in a prospective cohort of women,

Table 4
Association of rs12951053 and rs8073498 with BCC risk by subgroups of environmental and host factors.

	Cases			Controls			Per allele		*P* value	AA vs. CC		*P* value	AA vs. (AC + CC)		*P* value	*P* trend
	AA	AC	CC	AA	AC	CC	OR[a]	95% CI[a]		OR[a]	95% CI[a]		OR[a]	95% CI[a]		
rs12951053																
Sun-exposure ($P_{heterogeneity}$ = 0.0187)[b]																
>2.4	120	11	0	108	30	0	0.28	(0.12–0.62)	**0.002**							
2.4–3.5	96	17	2	104	9	2	1.91	(0.77–4.77)	0.16	0.62	(0.07–5.52)	0.67	1.66	(0.71–3.89)	0.25	0.19
3.6–4.5	105	19	1	83	14	3	1.11	(0.51–2.42)	0.78	0.33	(0.03–3.42)	0.35	0.99	(0.47–2.06)	0.97	0.58
≥4.5	119	32	0	142	26	2	1.36	(0.75–2.48)	0.31				1.28	(0.71–2.31)	0.41	0.44
Skin response to sun effect[c]																
Blistered/burnt	147	36	1	122	15	1	1.95	(0.99–3.81)	0.05	0.76	(0.05–12.69)	0.85	1.87	(0.97–3.59)	0.06	0.06
Mild burn	144	21	2	125	27	5	0.69	(0.36–1.33)	0.27	0.37	(0.06–2.30)	0.29	0.65	(0.35–1.20)	0.17	0.08
Tanning/no change	138	22	0	183	35	1	0.83	(0.46–1.52)	0.55				0.81	(0.44–1.46)	0.48	0.41
Risk[c]																
Low	140	22	0	189	37	2	0.81	(0.45–1.46)	0.48				0.76	(0.42–1.38)	0.37	0.26
Medium	63	12	1	67	17	1	0.81	(0.35–1.90)	0.63	1.57	(0.08–29.54)	0.76	0.85	(0.37–1.93)	0.69	0.57
High	237	45	2	181	25	4	1.22	(0.71–2.11)	0.47	0.31	(0.05–1.92)	0.21	1.10	(0.65–1.86)	0.72	0.68
Age[d]																
<55	82	6	1	139	23	4	0.43	(0.16–1.15)	0.09	0.35	(0.04–3.43)	0.37	0.42	(0.17–1.04)	0.06	0.07
55–63	87	25	0	113	13	1	2.67	(1.27–5.64)	0.01				2.51	(1.2–5.22)	0.01	0.043
64–72	135	26	0	102	21	1	0.93	(0.49–1.77)	0.83				0.88	(0.47–1.66)	0.69	0.60
>72	136	22	2	83	22	1	0.61	(0.31–1.22)	0.16	1.10	(0.08–15.23)	0.95	0.63	(0.33–1.24)	0.18	0.22
Complexion[c]																
Light	235	40	2	174	31	4	0.87	(0.51–1.48)	0.60	0.27	(0.04–1.73)	0.17	0.80	(0.48–1.34)	0.39	0.46
Medium	191	36	1	211	41	2	0.96	(0.58–1.60)	0.88	0.73	(0.06–8.73)	0.80	0.95	(0.58–1.57)	0.85	0.77
Dark	13	3	0	51	7	1	1.90	(0.41–8.75)	0.41				1.62	(0.36–7.27)	0.53	0.76
p53rs8073498																
Sun-exposure[b] ($P_{heterogeneity}$ = 0.4322)																
>2.4	51	59	20	44	78	17	0.59	(0.33–1.05)	0.07	0.77	(0.34–1.76)	0.540	0.63	(0.36–1.08)	0.09	0.59
2.4–3.5	39	58	18	39	58	16	1.00	(0.54–1.84)	0.10	0.95	(0.40–2.25)	0.911	0.99	(0.55–1.77)	0.97	0.82
3.6–4.5	38	69	18	48	34	17	2.83	(1.50–5.35)	**0.001**	1.39	(0.61–3.18)	0.431	2.32	(1.29–4.16)	0.005	0.10
≥4.5	56	61	32	67	78	23	0.93	(0.56–1.55)	0.79	1.57	(0.80–3.08)	0.185	1.08	(0.67–1.73)	0.75	0.21
Skin response to sun effect[c]																
Blistered/burnt	63	88	30	52	65	21	1.05	(0.63–1.74)	0.86	1.09	(0.54–2.18)	0.816	1.06	(0.65–1.71)	0.82	0.59
Mild burn	61	86	21	50	80	24	0.89	(0.54–1.49)	0.67	0.69	(0.33–1.44)	0.323	0.85	(0.52–1.37)	0.50	0.35
Tanning/no change	57	68	34	92	98	28	1.24	(0.77–2.00)	0.37	1.68	(0.90–3.13)	0.105	1.35	(0.87–2.1)	0.18	0.05
Risk[c]																
Low	57	70	34	94	105	28	1.19	(0.75–1.90)	0.47	1.72	(0.92–3.20)	0.088	1.31	(0.85–2.03)	0.22	0.04
Medium	30	38	9	29	41	13	1.01	(0.50–2.03)	0.99	0.58	(0.20–1.65)	0.309	0.89	(0.46–1.74)	0.73	0.45
High	97	139	45	75	102	32	0.98	(0.65–1.48)	0.91	1.07	(0.60–1.89)	0.824	1.00	(0.67–1.48)	0.99	0.74
Age[d]																
<55	28	44	15	67	76	23	1.56	(0.85–2.86)	0.15	1.34	(0.59–3.04)	0.488	1.50	(0.85–2.65)	0.17	0.21
55–63	40	54	18	49	64	12	0.97	(0.55–1.72)	0.92	1.77	(0.74–4.23)	0.197	1.10	(0.64–1.88)	0.74	0.25
64–72	58	80	24	40	64	19	0.83	(0.49–1.42)	0.50	0.82	(0.39–1.73)	0.607	0.83	(0.5–1.38)	0.47	0.63
>72	58	69	31	42	44	19	1.13	(0.64–2.01)	0.68	1.26	(0.61–2.59)	0.539	1.17	(0.69–1.98)	0.57	0.60
Complexion[b]																
Light	105	129	42	79	98	30	1.04	(0.69–1.58)	0.84	0.99	(0.56–1.77)	0.979	1.03	(0.70–1.52)	0.88	0.89
Medium	74	111	41	92	124	36	1.10	(0.73–1.66)	0.65	1.28	(0.73–2.25)	0.390	1.14	(0.77–1.69)	0.51	0.23
Dark	5	7	4	26	26	7	1.46	(0.40–5.37)	0.57	2.58	(0.51–12.92)	0.250	1.74	(0.52–5.75)	0.37	0.19

[a] OR: odds ratio; CI: confidence interval. Study-wise significant associations ($P < 0.0042$) are written in bold, associations with $P < 0.05$ but not reaching the threshold for multiple comparison are underlined.
[b] Adjusted for age, gender, risk (which consists of the combination of skin complexion and skin response to sun exposure) and nationality.
[c] Adjusted for age, gender and nationality.
[d] Adjusted for gender, risk and nationality.

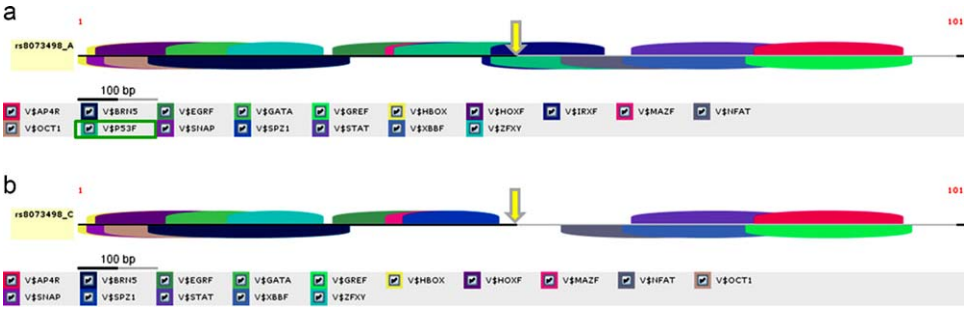

Fig. 2. Output of MatInspector software. The yellow arrow indicates the position of rs8073498 SNP. The C allele abolishes recognition motifs for p53 (in a green frame). The recognition motif is predicted with a matrix similarity of 0.94 and 0.98 (a. output for A allele, b. output for C allele). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

showed no association between one SNP in *POMC* gene (rs1042571) and BCC risk [18]. Our results show no association with the p53 codon 72 polymorphism, and confirm lack of association with *POMC* polymorphisms covering the whole gene, including rs1042571. Nan et al. [36] tested also an interaction analysis between p53 codon 72 polymorphism and MC1R variants. The authors found that there was an association between the two genes and melanoma risk but not for BCC risk. Our results on the interactions between the two gene variants confirm in a bigger independent population their null finding.

Sun exposure is the major etiological factor in the genesis of BCC; however, many studies have suggested that risk may involve an interplay between genetic (SNPs), host (skin complexion, skin response to sun exposure) and environmental (sun exposure) factors [2]. Thus we have thoroughly analyzed all the possible interactions and associations between the selected polymorphisms and known risk factors for BCC. Analyzing the data in subgroups stratifying for age, gender and the host factors previously mentioned, we found that two polymorphisms were associated with BCC risk. We found that, in the group of subjects with lower sun exposure during the summer, carriers of one copy of the C allele of the rs12951053 had a decreased risk of BCC and subjects with intermediate-high sun exposure had an increased risk of BCC in carriers of one copy of the C allele of SNP rs8073498, although the heterogeneity test in this subgroup analysis was not significant. The rs12951053 polymorphism has been found associated with increased risk in other cancer types [37–39]. On the other hand, we observed it to be associated with a decrease in BCC risk. p53 is known to act in many different ways, depending on the tissue and environmental stimuli; in this specific case its function is likely related with UV response. In different cancer types the major function can be a different one with different regulations. *In silico* analysis predicts that several variants in moderate LD ($0.5 < r^2 < 0.7$) with *TP53* rs12951053 can affect a transcription factor binding site; in addition, rs2287498, which is in the flanking gene *WDR79* and in LD with rs12951053 ($r^2 = 0.62$), is predicted to affect function at a splice site [39]. The mRNA encoding this protein plays a critical role in the regulation of p53 expression at the post-transcriptional level; it is involved both in maintaining basal p53 mRNA levels and in p53 induction upon DNA damage [40]. So far there is no published association between genotypes in rs8073498 and cancer risk. We assessed the putative effect of the C allele by the MatInspector program, which predicts that this variant may abolish recognition motifs for p53 itself (Fig. 2).

In this study we had more than 80% power to detect a possible association with a minimum OR of 1.28 for a SNP with minor allele frequency of 0.45 in the controls assuming alpha = 0.05, two-sided test and a codominant model. In conclusion, we have observed that the interplay of sun exposure and one polymorphism in *TP53* gene association could modulate the risk of BCC. However these results have to be taken with caution due to the relatively large number of comparisons done and have to be replicated in a larger independent study.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jdermsci.2011.03.006.

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