EUROPEAN JOURNAL OF CANCER 47 (2011) 2618–2625

available at www.sciencedirect.com

ScienceDirect

journal homepage: www.ejconline.com

Genetic polymorphisms in DNA repair and oxidative stress pathways associated with malignant melanoma susceptibility

Maider Ibarrola-Villava [a], *Maria Peña-Chilet* [a], *Lara P. Fernandez* [b], *Jose A. Aviles* [c], *Matias Mayor* [d], *Manuel Martin-Gonzalez* [e], *Cristina Gomez-Fernandez* [d], *Beatriz Casado* [d], *Pablo Lazaro* [c], *Ana Lluch* [a], *Javier Benitez* [f], *Rafael Lozoya* [g], *Enrique Boldo* [g], *Angel Pizarro* [d], *Conrado Martinez-Cadenas* [h], *Gloria Ribas* [a,*]

[a] *Department of Haematology and Medical Oncology, Fundacion Investigacion Hospital Clinico Universitario-INCLIVA, Valencia, Spain*
[b] *Institute of Biomedical Research, Alberto Sols, CSIC, University Autonoma of Madrid, Madrid, Spain*
[c] *Department of Dermatology, Gregorio Marañon Hospital, Madrid, Spain*
[d] *Department of Dermatology, La Paz Hospital, Madrid, Spain*
[e] *Department of Dermatology, Ramon y Cajal Hospital, Madrid, Spain*
[f] *Human Genetics Group, Spanish National Cancer Centre, CNIO, Madrid, Spain*
[g] *Surgical Oncology Unit, Castellon Province Hospital, Castellon, Spain*
[h] *Laboratory of Molecular Biopathology, Castellon Province Hospital, Spain*

ARTICLE INFO

Article history:
Available online 9 June 2011

Keywords:
DNA repair
Oxidative stress
Polymorphism
Melanoma
Susceptibility

ABSTRACT

Background: Base excision repair (BER) and nucleotide excision repair (NER) pathways eliminate a wide variety of DNA damage, including UV photoproducts. The ability of each individual to repair DNA damage following different causes might explain at least in part the variability in cancer susceptibility. Moreover, inflammatory response to UV exposure may further contribute to skin carcinogenesis by oxidative stress mechanisms. Single nucleotide polymorphisms in genes encoding various DNA-repair enzymes and oxidative stress factors may be candidate low-penetrance variants with a role in susceptibility to different cancers, particularly in those with aetiologies linked to environmental exposure, such as malignant melanoma (MM).

Methods: In this case–control study, 684 Spanish sporadic MM patients and 406 cancer-free control subjects were included and the role of 46 polymorphisms belonging to 16 BER and NER genes as well as 11 genes involved in oxidative stress processes were investigated.

Results: One polymorphism was identified to be individually associated with MM in the Spanish population. The variant was found in the *NOS1* oxidative stress gene (rs2682826; *p-value* = 0.01). These results suggest a putative role of oxidative stress processes in the genetic predisposition to melanoma.

Conclusion: To the authors' knowledge, this is the largest DNA repair-related SNP study in melanoma risk conducted in the Spanish population up to now. Furthermore, it also represents a comprehensive genetic study of several oxidative stress polymorphisms tested in relation to MM susceptibility.

* *Corresponding author:* Address: Department of Haematology and Medical Oncology, Fundación Investigación Hospital Clínico, Av. Blasco Ibañez 17, 46010, Valencia, Spain. Tel.: +34 963862894; fax: +34 963987860.
E-mail address: gribasdespuig@gmail.com (G. Ribas).

0959-8049/$ - see front matter
doi:10.1016/j.ejca.2011.05.011

1. Introduction

Malignant melanoma (MM) represents aggressive neoplasm of melanocytes and its frequency is rapidly increasing among Caucasian populations.[1] The aetiology of MM remains unclear but it is known that both genetic and environmental factors influence the development of sporadic disease.[2] The key environmental risk factor is exposure to the ultraviolet (UV) component in sunlight, which causes various kinds of DNA damage, including bulky lesions and oxidative damage, which may lead to mutations if not repaired efficiently. Thus, variations in genes implicated in nucleotide excision repair, base excision repair and oxidative stress pathways may be associated with susceptibility to MM.[3]

The nucleotide excision repair (NER) pathway is one of the most versatile and best studied DNA repair systems and it deals with the main types of UV-induced DNA damage. Specifically, NER genes remove bulky DNA lesions, such as pyrimidine dimers caused by UV light, bulky adducts induced by chemical carcinogens, and other helix-distorting DNA lesions.[4] Since DNA repair is central to maintaining genomic integrity in the skin,[4,5] polymorphisms in the DNA repair genes may contribute to variations in DNA repair capacity in the general population and may affect genetic susceptibility to cancer. Individuals with inherited defects in NER have low repair levels of UV-induced DNA lesions and are at a high risk of skin cancers, including melanoma. Although several previous studies have investigated the association between polymorphisms in NER genes and risk of melanoma, most of the study sizes were relatively small, and the results were not consistent.[6–12]

The base excision repair (BER) pathway is responsible for repair of oxidative DNA damage and single-strand breaks generated indirectly by UV radiation.[13,14] It has been shown that modulation of BER alters cellular sensitivity to UVA but not to UVB.[15] The BER pathway, which possibly handles the largest number of cytotoxic and mutagenic base lesions, specifically removes alterations of a single base that has been methylated, oxidised or reduced and thus rectifies single-strand interruptions in DNA.[16] Genetic polymorphisms have been identified in several BER genes and studies suggest that some of these polymorphisms may be associated with cancer risk.[17–20]

Furthermore, UV radiation can indirectly induce oxidative stress in the skin via reactive oxygen species (ROS) generated after the absorption of light energy by cellular chromophores. Moreover, inflammatory response to UV exposure may further contribute to skin carcinogenesis by oxidative stress mechanisms.[21] In addition, UV radiation can alter antioxidant and other related enzymes, and impair the antioxidant defence in the skin.[21,22] ROS can damage DNA in the form of mutations, deletions, gene amplification and rearrangements. As a result, oxidative stress has already been implicated in skin carcinogenesis.[21,23]

In this context, DNA repair and oxidative stress genes may be involved in MM susceptibility. For this reason, we investigated, for the first time, the role of polymorphisms in these genes in MM predisposition in the Spanish population.

2. Patients and methods

2.1. Study subjects and data collection

A total of 684 consecutive and non-related MM cases were recruited from September 2004 to July 2010 at the Departments of Dermatology of four Spanish hospitals: Gregorio Marañon, La Paz, Ramon y Cajal in Madrid and Castellon Province in Castellon. A total of 406 volunteer cancer-free controls were recruited from 2004 to 2010 at the Madrid College of Lawyers, Gregorio Marañon Hospital and Castellon Province Hospital. All participants were Caucasians of Spanish origin.

A standardized questionnaire was used to collect information on pigmentation characteristics, such as eye, hair and skin colour, number of naevi, presence of solar lentigines, childhood sunburns and sun exposure habits. Fitzpatrick's classification of skin type, tumour localization, Breslow index (depth index) and personal or family history of cancer were also included in the questionnaire (Supplementary Table 1). All study subjects gave written informed consent and the study was approved by the Ethics Committee of Gregorio Marañon Hospital and University Clinic Hospital of Valencia.

Genomic DNA from cases and controls was isolated from peripheral blood lymphocytes and diluted to a final solution of 50 ng/μl. This was done by using the MagNA Pure LC Instrument according to the manufacturer's protocol (Roche Molecular Biochemicals AQ2, Mannheim, Germany), or by the DNAzol procedure (Invitrogen, Eugene, OR, USA). Some samples were extracted using the traditional saline method. DNA concentration was quantified in samples prior to genotyping by using Quant-iT PicoGreen dsDNA Reagent (Invitrogen, Eugene, OR, USA) and NanoDrop (NanoDrop Technologies, Wilmington, DE, USA). Genomic DNA was amplified using the GenomiPhi™ DNA Amplification Kit (GE Healthcare Bio-Sciences AB, Uppsala, Sweden).

2.2. SNP selection

Two public databases were used to collect information about single nucleotide polymorphisms (SNPs) in BER/NER and oxidative stress pathway genes: NCBI (http://www.ncbi.nlm.nih.gov) and HapMap (http://www.hapmap.org). SNPs selected were located in exons, in putative promoter regions or had been reported to be associated with cancer in previous studies. Forty-seven SNPs were initially selected and sent to be designed by Sequenom platform (30 SNPs from 16 BER/NER pathway genes and 17 SNPs from 11 oxidative stress pathway genes were finally selected). Finally, 35 assays were successfully genotyped using two plexes with the Sequenom platform. The 11 SNPs unsuccessfully designed or genotyped by Sequenom technology were subsequently redesigned using Taqman or Kaspar assays. No methodology was capable of genotyping rs2279744, which was finally discarded. All SNPs had a minor allele frequency (MAF) greater than or equal to 5%. Details of the genes and SNPs studied, including MIM code, location, encoded protein, gene function, nucleotide changes and context sequence are provided in Supplementary Table 2.

2.3. Genotyping reaction

2.3.1. Sequenom assay

Assays for 35 SNPs were genotyped by using the Sequenom MassARRAY Assay Design software version 3.0.0 (Sequenom Inc., San Diego, CA, USA). Assay primers will be supplied upon request. One sample duplicate and one father–mother–child trio were included across the plates to assess genotyping accuracy. SNPs were genotyped using iPLEX™ chemistry on a MALDI-TOF Mass Spectrometer (Sequenom Inc., San Diego, CA, USA). PCR was performed according to the manufacturer's instructions. Assays either with failed designs or without Sequenom genotyping results were redesigned using either TaqMan or Kaspar probes.

2.3.2. TaqMan assay

A total of 8 SNPs were genotyped using TaqMan technology, 6 additional SNPs plus 2 failed Sequenom assays (rs9350, *EXO1*; rs1042522, *P53*; rs1800975, *XPA*; rs1799793, *XPD*; rs1799782, *XRCC1*; rs861539, *XRCC3*; rs1052133, *OGG1* and rs1041740, *SOD1*). The PCR was carried out according to the manufacturer's instructions. After PCR, the genotype of each sample was automatically determined by measuring allele-specific final fluorescence in the ABI Prism 7900HT Detection System, using the SDS 2.3 software for allele discrimination (Applied Biosystems, Foster City, CA, USA). As a quality control measure, one sample duplicate and a non-template sample were included in each 96-well plate. Genotypes were scored by two different personnel in the laboratory. No discrepancies were observed.

2.3.3. Kaspar assay

Three SNPs (rs1805388, *LIG4*; rs1050450, *GPX1* and rs7855, *SOD2*) were genotyped using KASPAR SNP Genotyping System (KBiosciences, Hoddesdon, UK). Assay primers, probes and PCR conditions can be provided upon request. The PCR was carried out according to the manufacturer's instructions. Measurement was done in the ABI Prism 7900HT Detection System, using the SDS 2.3 software for allele discrimination (Applied Biosystems, Foster City, CA, USA). As a quality control measure, one sample duplicate and a non-template sample per 96-well plate were included. Genotypes were scored by two different personnel in the laboratory and no discrepancies were observed.

2.4. Statistical analysis

For all polymorphisms studied, Fisher's exact test was used both to test for deviations from Hardy–Weinberg equilibrium (HWE) in controls and to compare allele counts between cases and controls. Genotype-related odds ratios (ORs), their corresponding 95% confidence intervals (CIs), and associated *p*-values were estimated via unconditional logistic regression. Analyses of Sequenom, TaqMan and Kaspar SNPs were performed using the SNPator package (www.snpator.com) and SPSS v17.

Multivariate logistic regression was also applied, including eye colour (black/brown, blue/green), hair colour (black/brown, blond/red), skin colour (fair, brown), solar lentigines (yes, no) and childhood sunburns (yes, no) as covariables.

Associations between the genotyped BER/NER and oxidative stress pathway genes and various pigmentation characteristics and sun sensitivity were assessed via logistic regression. This was done for cases and controls pooled together for eye colour (blue/green *versus* brown), hair colour (blond/red *versus* brown/black), skin colour (fair *versus* brown), number of naevi ($\geqslant$50 *versus* <50), presence of lentigines (yes *versus* no) and childhood sunburn (yes *versus* no) as the outcome variables. The SNPator package and SPSS v17 were used to carry out these analyses.

3. Results

3.1. Allelic distribution of DNA repair and oxidative stress polymorphisms

Allele frequencies for each SNP and *p*-values for their comparison between 684 MM cases and 406 individual controls are detailed in Table 1, along with *p*-values for test of departure from HWE among controls.

Based on unadjusted *p*-values, we observed evidence of differences in allele frequency for one SNP in the *NOS1* gene (rs2682826, *p*-value = 0.01), implicated in the oxidative stress pathway. We did not observe differences in minor allele frequencies between cases and controls for any other SNP.

Each polymorphism was checked for HWE in cases and controls. All were found to be in equilibrium with the exception of *NOS1* rs2682826 (*p*-values = 0.01). However, use of the Bonferroni correction for the 46 tests would indicate that *p*-value < 0.001 was needed to reject HWE. Re-examination of the original results for these polymorphisms revealed no genotyping errors, so it seems likely that these results were the consequence of chance events.

3.2. Association between genotypes and melanoma risk

The implication of these DNA repair and oxidative stress genes in melanoma was further investigated by comparing the genotypic distributions of all SNPs studied. The estimated ORs and associated *p*-values are shown in Table 2.

One SNP was found to be associated with MM susceptibility. This SNP implicated in oxidative stress processes, rs2682826 in *NOS1* (*p*-value = 0.01), is associated with MM susceptibility in a protective way. No association remained statistically significant for any other SNP.

3.3. Association between genotypes and phenotypic characteristics

We assessed whether DNA repair and oxidative stress polymorphisms were associated with various phenotypic characteristics. We observed evidence of association for several genes. However, only one SNP, rs3218536, located in the *XRCC2* gene showed strong association in relation to different phenotypic traits. This SNP corresponds to a non-synonymous amino acid change, p.Arg188His. The His188 homozygote is associated with light hair colour (OR per allele, 7.22; 95% CI, 2.26–23.04; *p*-value = 0.0001), light skin colour (OR per allele, 4.39; 95% CI, 0.96–20.13; *p*-value = 0.038), presence of solar lentigines (OR per allele, 8.07; 95% CI, 1.03–63.35;

Table 1 – Allelic frequencies in Spanish cases and controls.

Gene	SNP	Nucleotide change	PHWE	MAF Controls	MAF Cases	*p*-value
BER/NER pathway						
ADPRT/PARP1	rs1136410	c.2285T>C	0.90	0.13	0.12	0.33
APE1/APEX1	rs1130409	c.444T>G	0.97	0.45	0.48	0.22
ERCC1	rs11615	c.354T>C	0.39	0.36	0.38	0.31
ERCC6	rs4253211	c.3689G>C	0.59	0.07	0.08	0.18
	rs2228526	c.3289A>G	0.51	0.22	0.23	0.77
	rs2228527	c.3637A>G	0.41	0.23	0.23	0.82
EXO1	rs9350	c.2270C>T	0.87	0.16	0.16	0.86
	rs1776148	c.2010G>A	0.88	0.35	0.36	0.82
	rs1047840	c.1765G>A	0.41	0.41	0.37	0.10
HR23B/RAD23B	rs1805329	c.746C>T	0.32	0.15	0.14	0.53
LIG4	rs1805386	c.1704T>C	0.78	0.15	0.16	0.66
	rs1805388	c.26C>T	0.77	0.14	0.14	0.81
TP53	rs1042522	c.215G>C	0.99	0.26	0.24	0.14
XPA	rs1800975	c.-4A>G	0.62	0.32	0.28	0.08
XPC	rs2228000	c.1496C>T	0.32	0.27	0.27	0.92
	rs2228001	c.2815C>A	0.08	0.41	0.44	0.20
XPD/ERCC2	rs238406	c.468A>C	0.95	0.49	0.48	0.76
	rs1052555	c.2133C>T	0.07	0.31	0.33	0.38
	rs13181	c.2251A>C	0.10	0.34	0.36	0.45
	rs1799793	c.934G>A	0.55	0.32	0.34	0.44
XPF/ERCC4	rs1800067	c.1244G>A	0.50	0.12	0.11	0.42
	rs1799797	g.4980T>A	0.97	0.31	0.31	0.92
	rs1799801	c.2505T>C	0.84	0.33	0.33	0.98
XPG/ERCC5	rs17655	c.3310G>C	0.90	0.25	0.27	0.34
XRCC1	rs1799782	c.580C>T	0.14	0.06	0.07	0.32
	rs25487	c.1196A>G	0.79	0.40	0.39	0.66
XRCC2	rs3218536	c.563G>A	0.84	0.11	0.11	0.72
	rs3218557	g.12939069T>C	0.62	0.45	0.45	0.94
XRCC3	rs861539	c.722C>T	0.96	0.40	0.38	0.27
Oxidative Stress pathway						
CAT	rs1001179	g.4760C>T	0.89	0.23	0.22	0.88
COMT	rs4680	c.472G>A	0.84	0.47	0.46	0.59
GPX1	rs1050450	c.599C>T	0.69	0.34	0.34	0.79
NOS1	rs2682826	c.*276C>T	**0.01**	0.33	0.27	**0.01**
NOS2	rs2297518	c.1823C>T	0.14	0.20	0.20	0.88
NOS3	rs1799983	c.894T>G	0.15	0.38	0.39	0.79
OGG1	rs1052133	c.977C>G	0.25	0.21	0.21	0.85
	rs125701	g.3851G>A	0.33	0.13	0.15	0.16
PON1	rs662	c.575A>G	0.50	0.30	0.28	0.46
	rs854560	c.163T>A	0.13	0.38	0.39	0.99
SOD1	rs1041740	c.357+474C>T	0.44	0.33	0.35	0.26
	rs2070424	c.240-251A>G	0.91	0.07	0.06	0.31
SOD2	rs4880	c.47T>C	0.92	0.49	0.48	0.18
	rs5746136	c.*441G>A	0.36	0.30	0.32	0.49
	rs7855	c.*48T>C	0.60	0.06	0.06	0.59
SOD3	rs2536512	c.172G>A	0.22	0.41	0.39	0.43
	rs699473	g.4719C>T	0.64	0.37	0.37	0.94

BER; base excision repair. NER; nucleotide excision repair. HWE. Hardy–Weinberg equilibrium.
Sequenom analysis; cases *N* = 599 and controls *N* = 379. TaqMan/Kaspar analysis; cases *N* = 684 and controls *N* = 406.
Bold indicates statistically significant results.

p-value = 0.018) and high naevus count (OR per allele, 3.62; 95% CI, 1.05–12.56; *p*-value = 0.030) (data shown in Table 3). Spurious phenotypic association effects are detected in 8 additional genes, although data are not as consistent as for rs3218536 (data not shown).

3.4. *Multivariate analysis*

We considered blue/green eye colour, blond/red hair colour, fair skin colour, solar lentigines and childhood sunburns as confounders in a multivariate model. The *NOS1* rs2682826 SNP retained statistical significant results even when adjusted for all potential confounders (OR, 0.61; 95% CI, 0.45–0.84; *p*-value = 0.02) (see Table 4).

4. Discussion

Genome integrity is continuously threatened by both endogenous and exogenous factors and is maintained by a complex network of more than 150 DNA repair-related proteins.[4]

Table 2 – Genotypic frequencies across BER/NER and oxidative stress pathway genes and assessment of individual associations with malignant melanoma.

Gene	SNP	Cases		Controls		OR (95% CI)	*p*-value
		Heterozygotes *n* (%)	Minor homozygotes[a] *n* (%)	Heterozygotes *n* (%)	Minor homozygotes[a] *n* (%)		
BER/NER pathway							
ADPRT/PARP1	rs1136410	114 (19.0)	13 (2.2)	85 822.4)	7 (1.8)	0.88 (0.67–1.15)	0.36
APE1/APEX1	rs1130409	301 (50.3)	132 (22.0)	185 (48.8)	75 (19.8)	1.11 (0.93–1.34)	0.25
ERCC1	rs11615	281 (46.9)	86 (14.4)	182 (48.0)	45 (11.9)	1.10 (0.90–1.33)	0.35
ERCC6	rs4253211	94 (15.7)	4 (0.7)	51 (13.5)	0 (0.0)	1.30 (0.91–1.85)	0.15
	rs2228526	215 (35.9)	30 (5.0)	137 (36.1)	16 (4.2)	1.03 (0.82–1.28)	0.80
	rs2228527	219 (36.6)	30 (5.0)	140 (36.9)	16 (4.2)	1.02 (0.82–1.27)	0.85
EXO1	rs9350	186 (27.2)	13 (1.9)	99 (24.4)	10 (2.5)	0.98 (0.76–1.25)	0.86
	rs1776148	293 (48.9)	67 (11.2)	171 (45.1)	48 (12.7)	1.02 (0.84–1.24)	0.85
	rs1047840	282 (47.1)	83 (13.9)	175 (46.2)	68 (17.9)	0.86 (0.72–1.04)	0.12
HR23B/RAD23B	rs1805329	153 (25.5)	5 (0.8)	103 (27.2)	4 (1.1)	0.92 (0.70–1.20)	0.52
LIG4	rs1805386	154 (25.7)	16 (2.7)	97 (26.5)	7 (1.7)	1.07 (0.83–1.38)	0.62
	rs1805388	163 (23.8)	11 (1.6)	98 (24.1)	7 (1.7)	0.97 (0.75–1.25)	0.81
TP53	rs1042522	244 (35.7)	35 (5.1)	156 (38.4)	28 (6.9)	0.86 (0.70–1.05)	0.13
XPA	rs1800975	175 (29.2)	28 (4.7)	116 (30.6)	20 (5.3)	0.84 (0.70–1.02)	0.08
XPC	rs2228000	227 (37.9)	49 (8.2)	158 (41.7)	23 (6.1)	1.01 (0.82–1.24)	0.93
	rs2228001	289 (48.2)	114 (19.0)	198 (52.2)	54 (14.2)	1.12 (0.92–1.34)	0.26
XPD/ERCC2	rs238406	306 (51.1)	135 (22.5)	185 (48.8)	89 (23.5)	0.98 (0.81–1.18)	0.82
	rs1052555	273 (45.6)	59 (9.8)	179 (47.2)	27 (7.1)	1.10 (0.90–1.35)	0.34
	rs13181	281 (46.9)	73 (12.2)	186 (49.1)	36 (9.5)	1.08 (0.89–1.32)	0.42
	rs1799793	312 (45.6)	73 (10.7)	182 (44.8)	39 (9.6)	1.08 (0.89–1.30)	0.44
XPF/ERCC4	rs1800067	117 (19.5)	7 (1.2)	87 (23.0)	3 (0.8)	0.88 (0.66–1.17)	0.37
	rs1799797	238 (39.7)	66 (11.0)	162 (42.7)	37 (9.8)	1.01 (0.83–1.22)	0.95
	rs1799801	246 (41.1)	75 (12.5)	170 (44.9)	41 (10.8)	1.00 (0.82–1.21)	0.98
XPG/ERCC5	rs17655	222 (37.1)	50 (8.3)	140 (36.9)	24 (6.3)	1.11 (0.90–1.36)	0.33
XRCC1	rs1799782	90 (13.1)	3 (0.4)	40 (9.9)	3 (0.7)	1.20 (0.84–1.72)	0.32
	rs25487	272 (45.4)	94 (15.7)	183 (48.3)	58 (15.3)	0.95 (0.79–1.15)	0.62
XRCC2	rs3218536	111 (18.5)	8 (1.3)	77 (20.3)	4 (1.1)	0.94 (0.70–1.25)	0.67
	rs3218557	270 (45.1)	136 (22.7)	181 (47.8)	79 (20.8)	1.00 (0.84–1.20)	0.99
XRCC3	rs861539	309 (45.2)	101 (14.8)	193 (47.5)	65 (16.0)	0.90 (0.76–1.08)	0.27
Oxidative stress pathway							
CAT	rs1001179	205 (34.2)	32 (5.3)	134 (35.4)	19 (5.0)	0.98 (0.79–1.22)	0.88
COMT	rs4680	290 (48.4)	125 (20.9)	184 848.6)	84 822.2)	0.95 (0.79–1.14)	0.59
GPX1	rs1050450	308 (45.0)	75 (11.0)	175 (43.1)	47 (11.6)	1.02 (0.85–1.23)	0.79
NOS1	rs2682826	236 (39.4)	45 (7.5)	189 (49.9)	27 (7.1)	**0.77 (0.62–0.94)**	**0.01**
NOS2	rs2297518	193 (32.2)	23 (3.8)	133 (35.1)	8 (2.1)	1.02 (0.81–1.29)	0.87
NOS3	rs1799983	264 (24.5)	100 (16.7)	190 (50.1)	48 (12.7)	1.03 (0.85–1.24)	0.79
OGG1	rs1052133	212 (31.0)	34 (5.0)	122 (20.2)	21 (5.2)	0.98 (0.79–1.21)	0.85
	rs125701	147 (24.5)	16 (2.7)	76 (20.0)	10 (2.6)	1.20 (0.93–1.56)	0.17
PON1	rs662	229 (38.2)	55 (9.2)	152 (40.1)	37 (9.8)	0.93 (0.77–1.13)	0.47
	rs854560	266 (44.4)	96 (16.0)	163 (43.0)	63 (16.6)	1.00 (0.83–1.20)	0.99
SOD1	rs1041740	302 (44.2)	87 (12.7)	170 (41.9)	47 (11.6)	1.10 (0.92–1.33)	0.27
	rs2070424	60 (10.0)	3 (0.5)	46 (12.1)	2 (0.5)	0.83 (0.60–1.20)	0.32
SOD2	rs4880	293 (48.9)	137 (22.9)	187 (49.3)	92 (24.3)	0.88 (0.74–1.06)	0.18
	rs5746136	262 (43.7)	59 (9.8)	150 (39.6)	39 (10.3)	1.07 (0.88–1.30)	0.50
	rs7855	79 (11.6)	0 (0.0)	49 (12.1)	1 (0.2)	0.90 (0.62–1.31)	0.58
SOD3	rs2536512	232 (38.7)	78 (13.0)	142 (37.5)	58 (15.3)	0.92 (0.76–1.13)	0.44
	rs699473	260 (43.4)	89 (14.9)	171 (45.1)	54 (14.2)	0.99 (0.82–1.20)	0.94

BER; base excision repair. NER; nucleotide excision repair.
OR, odds ratio per minor allele; CI, confidence interval.
Sequenom analysis; cases *N* = 599 and controls *N* = 379. TaqMan/Kaspar analysis; cases *N* = 684 and controls *N* = 406.
Bold indicates statistically significant results.
[a] Minor homozygotes are homozygotes for the minor allele.

Polymorphisms in several of these DNA repair-related genes have been found to be associated with risk of developing different tumour types, although results are not always unequivocal. Individual variation in BER/NER and oxidative stress genes is one of the host factors that may influence UV radiation-related cancer risk, such as melanoma.

In this case–control study, which included 684 sporadic melanoma patients and 406 cancer free control subjects, the

Table 3 – Associations between *XRCC2* SNP rs3218536 and phenotypic characteristics.

Characteristic	OR (95% CI)	*p*-value
Eye colour	2.94 (0.93–9.35)	0.0552
Hair colour	7.22 (2.26–23.04)	**0.0001**
Skin colour	4.39 (0.96–20.13)	**0.0379**
Solar lentigines	8.07 (1.03–63.35)	**0.0180**
Number of nevi	3.62 (1.05–12.56)	**0.0302**
Childhood sunburn	1.86 (0.56–6.24)	0.3041

OR, odds ratio per minor allele; CI, confidence interval.
Bold indicates statistically significant results.

Table 4 – Multivariate analysis of *NOS1* SNP rs2682826 with MM risk factors.

Risk factor	OR (95% CI)	*p*-value
Eye colour	0.88 (0.61–1.29)	0.53
Hair colour	3.78 (2.13–6.71)	$\mathbf{5.75 \times 10^{-6}}$
Skin colour	1.17 (0.84–1.62)	0.35
Solar lentigines	2.53 (1.85–3.47)	$\mathbf{8.08 \times 10^{-9}}$
Childhood sunburns	3.40 (2.47–4.68)	$\mathbf{7.95 \times 10^{-4}}$
NOS1 SNP rs2682826	0.61 (0.45–0.84)	**0.02**

Results marked in bold correspond to *p*-values lower than 0.05.
All risk factors listed were included together in a multivariate logistic regression analysis to estimate ORs, CIs and *p*-values.
OR, odds ratio per minor allele; CI, confidence interval.

role of 46 polymorphisms belonging to 16 BER and NER genes as well as 11 genes involved in oxidative stress processes was investigated. This is the largest DNA repair-related polymorphism study undertaken in the Spanish population so far. Furthermore, this study also represents a fairly comprehensive SNP analysis of oxidative stress pathway genes in relation to susceptibility to MM.

Reactive oxygen species and other free radicals can cause oxidative damage to all components of the cell and have been shown to be involved in a number of pathologic conditions including cancer.[24–28] The *NOS1* gene is located on chromosome 12q24.2 and consists of 29 exons and 28 introns, encompassing more than 160 kb of genomic DNA,[29] and is the main NO synthesising enzyme in the central nervous system.[30,31] The rs2682826 SNP is located in the 3' UTR of exon 29 of the *NOS1* gene and was selected as the tag SNP of one of the most frequent haplotypes. However, the most likely functional SNP certainly seems to be rs2682826, located close to several miRNAs binding sites in the gene's 3'UTR. Possibly, differences in protein translation might be elicited depending on the allele present in the mRNA of this gene. No other regulatory element close to this region seems to modulate this gene. We propose this SNP as a novel variant related to melanoma (*p*-value = 0.01) with significant effect even when potential covariable factors are included in a multivariate model (OR, 0.61; 95% CI, 0.45–0.84; *p*-value = 0.02). Furthermore, in a stratified analysis, rs2682826 was found to be significantly associated with melanoma among highly UV exposed subjects, with the OR lowering to 0.75 (95% CI, 0.57–0.98; *p*-value = 0.03). In addition, statistical differences are enhanced when only light skin colour individuals are analysed (OR, 0.71; 95% CI, 0.53–0.96; *p*-value = 0.02). A previous study by Li et al.[32] also suggested a significant association between the *NOS1* gene and melanoma predisposition, yet in this study the most associated SNP was located in the promoter region of the gene.[32]

This finding is indeed consistent with the rational that both genetic susceptibility and DNA damage through UV exposure are required for the onset of the disease.

In addition, a small study of 218 MM patients evaluating the role of two SNPs located in genes belonging to the oxidative stress pathway has been recently published.[33] Homozygotes for the minor allele of SNP rs1050450 (Leu198Leu), located at the *GPX1* gene, were found to be associated with MM predisposition, results that we could not replicate in our study.

Also recently, a study combining MM samples from Spain and Germany investigated the role of 13 SNPs in 8 DNA repair genes. After comparison of our data with that of a mixed population, our results were in concordance with the ones presented by Figl et al.[34] The only statistically significant SNP detected in this last study (rs861539, OR, 0.83; CI95%, 0.79–0.98; *p*-value = 0.03), located in the *XRCC3* gene, has also been analysed by us and we obtained a similar trend to signification (OR, 0.90; 95% CI, 0.76–1.08). When both datasets (1857 melanoma cases and 1674 control subjects) are analysed together, a combined significant *p*-value of 0.03 and an OR of 0.86 (95% CI, 0.75–0.98) is obtained. Thus, this SNP in *XRCC3* could also be another susceptibility gene involved in melanoma predisposition.

Further positive results obtained in two independent genetic studies by Li and colleagues[35,36] which linked *TP53* and a gene-gene interaction between *XRCC1* and *APE1* variants to melanoma predisposition were not replicated in our study.

The discrepancies in the findings observed in our study and in previous works might be explained by differences in the genetic background of different ethnic groups, by different carcinogen exposure or by different study sample sizes. Variants showing lack of association could have very small effects, undetectable to the power of this study.

Interestingly, when DNA repair and oxidative stress polymorphisms were assessed together with phenotypic characteristics, evidence of association for several genes was observed. However, only minor homozygotes for one SNP, rs3218536, located in the *XRCC2* gene, appeared to be strongly associated with several phenotypic traits. This SNP encodes a non-synonymous amino acid change, p.Arg188His. His188 homozygotes are described here for the first time associated with light phenotypes, presence of solar lentigines and increased number of naevi.

In summary, we conducted a MM susceptibility case–control study including 46 SNPs in 27 oxidative stress and DNA repair-related genes in 684 Spanish melanoma patients and 406 controls. To our knowledge, this work is the largest and more comprehensive sporadic MM susceptibility study in the Spanish population until now. One SNP, rs2682826, implicated in oxidative stress processes, was associated with MM susceptibility, emphasising the importance of oxidative stress genes in MM risk. Additionally, we propose rs861539, in *XRCC3*, as a good candidate for further replication in larger DNA melanoma collections.

Conflict of interest statement

None declared.

Acknowledgements

This study was supported by grants from the Ministerio de Salud Carlos III (ISCIII) (FI10-00405). M.I.-V. is funded by the Spanish Ministerio de Educación y Ciencia under a Grant FPI (BES-2008009234). G.R. is funded by the Ministerio de Salud Carlos III under a "Miquel Servet" contract (CP08-00069).

We thank Mariano Casado, Angeles de la Riva Grandal, the Madrid College of Lawyers and the patients from the Gregorio Marañon, La Paz, Ramon y Cajal and Castellon Province Hospitals. Sequenom genotyping was performed at the Spanish National Genotyping Centre (CeGen, Santiago). Genotyping with Taqman and Kaspar was performed at the Unidad Central de Investigacion Medica (UCIM) of the Facultad de Medicina from the U. Valencia.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ejca.2011.05.011.

REFERENCES

1. Saenz S, Conejo-Mir J, Cayuela A. Melanoma epidemiology in Spain. *Actas Dermosifiliogr* 2005;**96**(7):411–8.
2. Bataille V. Genetic epidemiology of melanoma. *Eur J Cancer* 2003;**39**(10):1341–7.
3. Bishop JN, Harland M, Bishop DT. The genetics of melanoma. *Br J Hosp Med (Lond)* 2006;**67**(6):299–304.
4. Wood RD, Mitchell M, Lindahl T. Human DNA repair genes, 2005. *Mutat Res* 2005;**577**(1–2):275–83.
5. Hoeijmakers JH. Genome maintenance mechanisms for preventing cancer. *Nature* 2001;**411**(6835):366–74.
6. Millikan RC, Hummer A, Begg C, et al. Polymorphisms in nucleotide excision repair genes and risk of multiple primary melanoma: the Genes Environment and Melanoma Study. *Carcinogenesis* 2006;**27**(3):610–8.
7. Blankenburg S, Konig IR, Moessner R, et al. Assessment of 3 xeroderma pigmentosum group C gene polymorphisms and risk of cutaneous melanoma: a case–control study. *Carcinogenesis* 2005;**26**(6):1085–90.
8. Han J, Colditz GA, Liu JS, Hunter DJ. Genetic variation in XPD, sun exposure, and risk of skin cancer. *Cancer Epidemiol Biomarkers Prev* 2005;**14**(6):1539–44.
9. Liu D, O'Day SJ, Yang D, et al. Impact of gene polymorphisms on clinical outcome for stage IV melanoma patients treated with biochemotherapy: an exploratory study. *Clin Cancer Res* 2005;**11**(3):1237–46.
10. Baccarelli A, Calista D, Minghetti P, et al. XPD gene polymorphism and host characteristics in the association with cutaneous malignant melanoma risk. *Br J Cancer* 2004;**90**(2):497–502.
11. Tomescu D, Kavanagh G, Ha T, Campbell H, Melton DW. Nucleotide excision repair gene XPD polymorphisms and genetic predisposition to melanoma. *Carcinogenesis* 2001;**22**(3):403–8.
12. Wei Q, Lee JE, Gershenwald JE, et al. Repair of UV light-induced DNA damage and risk of cutaneous malignant melanoma. *J Natl Cancer Inst* 2003;**95**(4):308–15.
13. Memisoglu A, Samson L. Base excision repair in yeast and mammals. *Mutat Res* 2000;**451**(1–2):39–51.
14. Nilsen H, Krokan HE. Base excision repair in a network of defence and tolerance. *Carcinogenesis* 2001;**22**(7):987–98.
15. Kim KJ, Chakrabarty I, Li GZ, et al. Modulation of base excision repair alters cellular sensitivity to UVA1 but not to UVB1. *Photochem Photobiol* 2002;**75**(5):507–12.
16. Parikh SS, Mol CD, Tainer JA. Base excision repair enzyme family portrait: integrating the structure and chemistry of an entire DNA repair pathway. *Structure* 1997;**5**(12):1543–50.
17. Hung RJ, Hall J, Brennan P, Boffetta P. Genetic polymorphisms in the base excision repair pathway and cancer risk: a HuGE review. *Am J Epidemiol* 2005;**162**(10):925–42.
18. Lockett KL, Hall MC, Xu J, et al. The ADPRT V762A genetic variant contributes to prostate cancer susceptibility and deficient enzyme function. *Cancer Res* 2004;**64**(17):6344–8.
19. Hu Z, Ma H, Chen F, Wei Q, Shen H. XRCC1 polymorphisms and cancer risk: a meta-analysis of 38 case–control studies. *Cancer Epidemiol Biomarkers Prev* 2005;**14**(7):1810–8.
20. Wilson 3rd DM, Bohr VA. The mechanics of base excision repair, and its relationship to aging and disease. *DNA Repair (Amst)* 2007;**6**(4):544–59.
21. Sander CS, Chang H, Hamm F, Elsner P, Thiele JJ. Role of oxidative stress and the antioxidant network in cutaneous carcinogenesis. *Int J Dermatol* 2004;**43**(5):326–35.
22. Fuchs J, Huflejt ME, Rothfuss LM, et al. Impairment of enzymic and nonenzymic antioxidants in skin by UVB irradiation. *J Invest Dermatol* 1989;**93**(6):769–73.
23. Nishigori C, Hattori Y, Toyokuni S. Role of reactive oxygen species in skin carcinogenesis. *Antioxid Redox Signal* 2004;**6**(3):561–70.
24. Olinski R, Gackowski D, Foksinski M, et al. Oxidative DNA damage: assessment of the role in carcinogenesis, atherosclerosis, and acquired immunodeficiency syndrome. *Free Radic Biol Med* 2002;**33**(2):192–200.
25. Wan J, Winn LM. In utero-initiated cancer: the role of reactive oxygen species. *Birth Defects Res C Embryo Today* 2006;**78**(4):326–32.
26. Lee KW, Lee HJ. Biphasic effects of dietary antioxidants on oxidative stress-mediated carcinogenesis. *Mech Ageing Dev* 2006;**127**(5):424–31.
27. Kawanishi S, Hiraku Y, Oikawa S. Mechanism of guanine-specific DNA damage by oxidative stress and its role in carcinogenesis and aging. *Mutat Res* 2001;**488**(1):65–76.
28. Guyton KZ, Kensler TW. Oxidative mechanisms in carcinogenesis. *Br Med Bull* 1993;**49**(3):523–44.
29. Hall AV, Antoniou H, Wang Y, et al. Structural organization of the human neuronal nitric oxide synthase gene (NOS1). *J Biol Chem* 1994;**269**(52):33082–90.
30. Snyder SH, Ferris CD. Novel neurotransmitters and their neuropsychiatric relevance. *Am J Psychiatry* 2000;**157**(11):1738–51.
31. Baranano DE, Ferris CD, Snyder SH. Atypical neural messengers. *Trends Neurosci* 2001;**24**(2):99–106.
32. Li C, Hu Z, Liu Z, et al. Polymorphisms of the neuronal and inducible nitric oxide synthase genes and the risk of cutaneous melanoma: a case–control study. *Cancer* 2007;**109**(8):1570–8.
33. He C, Qureshi AA, Han J. Polymorphisms in genes involved in oxidative stress and their interactions with lifestyle factors on skin cancer risk. *J Dermatol Sci* 2010;**60**(1):54–6.
34. Figl A, Scherer D, Nagore E, et al. Single-nucleotide polymorphisms in DNA-repair genes and cutaneous melanoma. *Mutat Res* 2010;**702**(1):8–16.

35. Li C, Chen K, Liu Z, et al. Polymorphisms of TP53 Arg72Pro, but not p73 G4C14>A4TA4 and p21 Ser31Arg, contribute to risk of cutaneous melanoma. *J Invest Dermatol* 2008;**128**(6):1585–8.

36. Li C, Liu Z, Wang LE, et al. Genetic variants of the ADPRT, XRCC1 and APE1 genes and risk of cutaneous melanoma. *Carcinogenesis* 2006;**27**(9):1894–901.