

INVESTIGATIVE REPORT

Polymorphisms of GSTM1 and GSTT1, Sun Exposure and the Risk of Melanoma: A Case-control Study

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Glutathione S-transferases (GSTs) are a family of enzymes that are known to play an important role in cellular protection against oxidative stress, including the oxidative stress caused by ultraviolet radiation. This study focused on the possible involvement of GSTM1 and GSTT1 polymorphisms in risk modulation of cutaneous melanoma. Within a case-control study, the presence of the null polymorphism at GSTM1 and GSTT1 was investigated in 188 cases of cutaneous melanoma and 152 controls. Information on socio-demographic characteristics, medical history, sun exposure and pigmented characteristics were collected for all subjects. Logistic regression was used to estimate odds ratio (OR) and 95% confidence intervals (CI). An interaction was suggested between the GSTM1 and GSTT1 “null” genotype and episodes of sunburn in childhood OR of interaction (1.65, 95% CI (95% CI) 0.27–9.94). The risk of melanoma among the subset of participants who reported sunburns in childhood and who had both null variants, was nine (OR 9.16; 95% CI 1.18–70.9). The results suggest that subjects carrying both GSTM1 and GSTT1 null polymorphisms and experiencing sunburns in childhood have an extremely high risk of melanoma. **Key words: GSTM1; GSTT1; sun exposure; melanoma**

(Accepted November 19, 2010.)

Acta Derm Venereol 2011; 91: 284–289.

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Melanoma is an increasingly common malignancy of melanocytes and is currently one of the most rapidly increasing cancers in Caucasian populations (1). Cutaneous melanoma may result from a multi-factorial process involving both genetic predisposition and exposure to environmental factors (2, 3). Although ultraviolet radiation (UVR) is the only well-established environmental risk factor for cutaneous melanoma, the relationship between sun exposure and melanoma risk

is very complex. For example, melanoma risk does not appear to increase with increasing sun exposure, and dose-response is an important criterion for causality (4). The lack of dose-response could partially be explained by the inter-individual variation in response to sun exposure due to different genotypes and/or different patterns of sun exposure.

Familial studies suggest a joint effect of genetics and intermittent sun exposure. Thomas et al. (5) suggested that subjects who are intermittently exposed to sun have more frequent BRAF mutations than do subjects who are chronically exposed. Chaudru et al. (6) showed that subjects with CDK2A mutations and sunburns are at a greater risk of melanoma than subjects with CDK2A mutations and no sunburns.

There are numerous mechanisms to protect human skin against DNA damage from sun exposure, such as increasing epidermal thickness, skin pigmentation, DNA repair mechanisms, apoptosis and, last but not least, antioxidant enzymes such as glutathione S-transferase (GST) enzymes (7). The GST supergene family currently comprises eight families of genes (μ , π , θ , α , σ , κ , ζ and ω) encoding enzymes involved in the detoxification of a variety of potentially mutagenic compounds, including products of UVR-induced oxidative stress (8). The homozygous deletion of GSTM1 or GSTT1 genes is a relatively common genetic variation in European populations, which results in lack of GSTM1 and GSTT1 proteins (9).

Many studies have established that polymorphisms in members of the GST gene family can be important determinants of cancer risk at the population level (9). However, the role of GSTM1 and GSTT1 polymorphisms in melanoma is not clear (10–14). Several studies have investigated the effects of GST variants on melanoma risk, but most of them addressed only the role of GSTM1 (10, 11, 13). Moreover, none of these studies investigated the possible interaction between sun exposure and GSTM1 and GSTT1 polymorphisms in determining cutaneous melanoma risk. Therefore, the objective of this study was to investigate the role of GSTM1 and GSTT1 polymorphisms and their interaction with sun exposure in risk modulation of cutaneous melanoma.

MATERIALS AND METHODS

Within a hospital-based case-control study, individual patterns at two polymorphic genes (GSTM1 and GSTT1) belonging to the GST supergene family were investigated. Eligible cases were subjects of European ethnicity aged 18 years or more, who were resident in the Lazio region and admitted to the hospitals IDI-San Carlo between May 2001 and May 2003. All cases had a new histologically-confirmed diagnosis of primary malignant cutaneous melanoma. The study was approved by the Istituto Dermatologico dell'Immacolata (IDI-IRCCS) ethics committee, and written consent was obtained from all participants.

Controls were selected from patients in the same hospital (IDI-San Carlo) during the study period, from the same geographical area and with no personal history of cancer and in the following hospital wards: General Surgery, Vascular Surgery, Orthopaedics, ENT, and General Medicine. A balance between diagnoses was maintained when sampling controls in order to minimize bias. The control subjects were frequency matched to cases by gender (1:1) and age (in 5-year age strata) to yield a sex- and age-distribution similar to that of cases.

Among the 455 subjects who consented to be re-contacted for future research, 5 had died and 340 (188 cases of cutaneous melanoma and 152 controls) donated blood. The response rates among cases and controls were 87% and 65%, respectively.

Exposure assessment

After obtaining informed consent, the participants were interviewed by two trained researchers using a structured questionnaire and then clinically examined for pigmented lesions. The questionnaire included information on socio-demographic characteristics, personal medical history, phenotypic traits (skin type, skin, hair and eye colour) and family history of skin cancer, lifetime sunlight exposure and sunburn history.

The pigmented lesions were identified and recorded according to a standard protocol (15). Acquired melanocytic naevi were defined as brown-to-black pigmented maculae or papulae of 2 mm or more in diameter, darker in colour than the surrounding skin and clinically different from freckles, lentigines, café-au-lait spots, seborrhoeic keratoses, and pigmented basal cell carcinomas. The number of naevi (> 2 mm) over the entire skin surface (except for the scalp, pubic region and perineum) were recorded and then classified as none, few (1–24), moderate (25–59), or many (≥ 60). Other skin and individual characteristics, such as freckles, solar lentigines, actinic keratosis and a past history of skin cancer, were also recorded. Solar lentigines were classified as: none, few (limited to a single body part), moderate (two body areas), or many (more than two body areas).

The Fitzpatrick system was used to classify skin photo-type (burning and tanning tendency) (16). Hair colour at 20 years of age was classified as red and blonde, light brown, dark brown and black. Eye colour was divided into three categories: blue, grey and green/light brown/dark brown and black. A skin, eye and hair colour chart was used to help define skin, hair and eye colour during the interview.

Sun exposure history included: the mean daily hour outdoors in three different life periods: < 12 years, 12–18 years and ≥ 19 years. Lifetime sun exposure was the sum of the mean hours outdoors during lifetime. Lifetime sun exposure was classified into tertiles (low: ≤ 26; medium: 27–36; high: ≥ 37 h) based on the controls distribution. Sunburn episodes (pain and erythema and/or blisters for more than 24 h) were classified into 2 categories (none; one or more sunburns in childhood).

GSTM1 and GSTT1 genotyping

Blood samples were taken, and sent on dry ice to the Central Laboratory of CONTARP-INAIL (Consulenza Tecnica Accertamento

Rischi e Prevenzione), where DNA extraction and genotyping analyses were performed. Individual genomic DNA was extracted and processed from whole blood using the QUIamp DNA Blood Mini Kit (Qiagen, Milano, Italy) following the manufacturer's instructions. Individual homozygous deletions at two polymorphic genes (GSTM1 and GSTT1) belonging to GST family were determined using polymerase chain reaction (PCR)-based assays. In total, 188 cases of cutaneous melanoma and 152 controls were genotyped for GSTM1 and GSTT1 genes. GSTM1 fragment was amplified from genomic DNA template using the primers G5: 5'-GAACTCCCTGAAAAGCTAAAGC-3' and G6: 5'-GTTGGGCTCAAATATACGGTGG-3' (15); GSTT1 fragment was amplified from genomic DNA template using the primers GSTT1-1: 5'-TTCCTTACTGGTCCCTCACATCTC-3' and GSTT1-2: 5'-TCACCGGATCATGGCCAGCA-3' (12). All PCR reactions were performed with a β-globin-positive internal control using the following primers: PC04: 5'-CAACTTCATCCACGTTACC-3' and GH20: 5'-GAAGAGCCAAGGACAGTTAC-3' (15). The PCR temperature cycling profiles consisted of one cycle at 94°C for 5 min, followed by 40 cycles of denaturation at 94°C for 1 min, annealing of primers at 55°C for 1 min, elongation of primers at 72°C for 1 min, and one final extension step at 72°C for 10 min. Conditions of use of Blue Taq Polymerase (Euroclone, Pavia, Italy) were provided by the manufacturer. Visualization of the amplification products was accomplished on 2–2.5% agarose gels, following standard procedures. DNAs from cases and controls were blinded and randomized on PCR plates; duplicate genotyping was performed for a randomly selected 20% of the total series for quality control. GSTM1 and GSTT1 individuals were dichotomized according to the absence or presence of the specific fragments. The "active" group was defined as those subjects with at least one active allele in either GSTM1 or GSTT1, whereas the "null" group was defined as those subjects with homozygous deletions in both GSTM1 and GSTT1.

Statistical analysis

Unconditional logistic regression was used for statistical analysis. Using the low-exposure category as a baseline, odds ratios (ORs) and 95% confidence interval (CI) for the intermediate and high exposure categories were calculated.

A univariate analysis was first conducted for GSTM1 and GSTT1 genotype and for all known risk factors for cutaneous melanoma (e.g. sun exposure, phenotypic traits, number of naevi). We then performed a multivariate analysis that considered GSTM1 and GSTT1 polymorphisms. Since it is difficult to separate the effects of pigmentary characteristics and ability to tan (skin photo-type), we avoided keeping variables in the model that were highly correlated and that did not contribute to the fit of the model. The likelihood ratio test was used to decide whether to keep each covariate in the model. Only those variables that made statistically significant contributions to the model were included ($p < 0.05$).

The following variables were considered in the regression models as potential confounders: sex, age, years of school attendance, hair colour, skin photo-type, solar lentigines, number of naevi and sunburn episodes in childhood. Effect modification by sex, age, phenotypic characteristics and sun exposure variables for GST variables was considered. We also conducted an interaction analysis between GSTM1 and GSTT1 null genotypes and sunburns, as well as separate multivariable analysis by sun exposure.

All analyses were performed using the statistical software package PC-STATA (Stata 9.0; StataCorp LP, College Station, Texas 77845, USA).

RESULTS

A total of 188 cases ($n = 86$; 45.7% males; $n = 102$; 54.3% females) and 152 controls ($n = 70$; 46.1% ma-

les; $n = 82$; 53.9% females) gave written consent, were interviewed, had a full skin examination and donated blood. The mean age of the cases and controls was 50.7 years (SD 13.4 years) and 48.0 years (SD 15.0 years), respectively (p -value=0.10).

Table SI (available from <http://www.medicaljournals.se/acta/content/?doi=10.2340/00015555-1078>) shows the socio-demographic, clinical and histological characteristics of the subjects participating in the study and the diagnosis of the controls. Cases were more highly educated than controls. The superficial spreading cutaneous melanoma was the most frequently seen (78.7%) and the trunk was the most common site (51.1%). The frequency of GSTM1 and GSTT1 null genotypes among the controls were 53.9% and 19.7%, respectively. Forty-four subjects (14.4% cases and 11.2% controls) were carriers of both GSTM1 and GSTT1 null genotypes.

Table I shows the association between pigmentary characteristics, sun exposure, GSTM1, GSTT1 polymorphisms and cutaneous melanoma. An increased cutaneous melanoma risk was found for subjects with light brown hair (OR 2.56; 95% CI 1.57–4.18) and for blonds and red heads (OR 5.11; 95% CI 2.31–11.3) vs. subjects with dark brown and black hair. Subjects with skin photo-type I and II had an increased risk (OR 2.46; 95% CI 1.55–3.88) compared with subjects with skin photo-type III and IV. The presence of freckles in childhood (OR 2.67; 95% CI 1.62–4.41), many solar lentigines (OR 4.16; 95% CI 1.80–9.61), common naevi (25–59 naevi, OR 3.21; 95% CI 1.76–5.84; ≥ 60 naevi, OR 5.78; 95% CI 3.20–10.5) were all associated with an increased cutaneous melanoma risk. Sunburn in childhood was associated with an increased risk of melanoma (OR 3.56; 95% CI 2.10–6.04). The presence of actinic keratosis lesions and/or a past history of non-melanocytic skin cancer, familial history of skin cancer and light-coloured eyes were associated with an increased risk, although with wide confidence intervals. Lifetime sun exposure was not associated with an increased risk of melanoma. GSTM1 and GSTT1 polymorphisms independently, were not associated with an increased risk of melanoma. However, when GSTM1 and GSTT1 null genotypes were combined an increase risk was observed, although with wide confidence intervals (OR 1.30; 95% CI 0.67–2.50) (Table I). After including other risk factors in the models, such as hair colour, skin photo-type, solar lentigines, number of naevi and sunburns in childhood, the risk of melanoma for the combined null genotype increased, without reaching the formal level of statistical significance, to 1.82 (95% CI, 0.83–4.01) (Table II). We also controlled, one at a time, in the model for eye colour, total sun exposure, family history of skin cancer, actinic keratosis and the presence of freckles. None of these variables made any statistical contribution to the model. Since an interaction was suggested between sunburns and GST null group (OR 1.65, 95% CI 0.27–9.94) we

Table I. Association between subjects' pigmentary characteristics, sun exposure, GSTM1, GSTT1 polymorphisms and cutaneous melanoma

	Cases ($n = 188$) ^a n (%)	Controls ($n = 152$) ^a n (%)	OR (95% CI) ^b
Hair colour			
Black/dark brown	71 (37.8)	97 (63.8)	1
Light brown	82 (43.6)	45 (29.6)	2.56 (1.57–4.18)
Fair/blond/red	35 (18.6)	10 (6.6)	5.11 (2.31–11.3)
Eye colour			
Black/dark brown	70 (37.2)	73 (48)	1
Light brown	40 (21.3)	24 (15.8)	1.87 (1.00–3.49)
Blue/grey/green	78 (41.5)	55 (36.2)	1.50 (0.92–2.44)
Skin photo-type^c			
III–IV	62 (33)	82 (54.3)	1
I–II	126 (67)	69 (45.7)	2.46 (1.55–3.88)
Family history of skin cancer			
No	177 (95.2)	144 (96.6)	1
Yes	9 (4.8)	5 (3.4)	1.31 (0.43–4.03)
Presence of freckles			
No	101 (56.7)	113 (76.9)	1
Yes	77 (43.3)	34 (23.1)	2.67 (1.62–4.41)
Common naevi (n)			
0–24	62 (33.0)	96 (63.2)	1
25–59	49 (26.1)	30 (19.7)	3.21 (1.76–5.84)
≥ 60	77 (41.0)	26 (17.1)	5.78 (3.20–10.5)
Solar lentigines			
None	10 (5.3)	21 (13.9)	1
Few/moderate	25 (13.4)	48 (31.8)	1.17 (0.47–2.94)
Many	152 (81.3)	82 (54.3)	4.16 (1.80–9.61)
Actinic keratosis/NMSC			
No	160 (87.9)	133 (91.7)	1
Yes	22 (12.1)	12 (8.3)	1.37 (0.63–2.99)
Sunburns in childhood			
No	77 (49)	103 (76.9)	1
Yes	80 (51)	31 (23.1)	3.56 (2.10–6.04)
Lifetime sun exposure (h)			
Low (≤ 26)	70 (40.7)	45 (30.6)	1
Medium (27–36)	50 (29.1)	61 (41.5)	0.51 (0.29–0.88)
High (≥ 37)	52 (30.2)	41 (27.9)	0.80 (0.44–1.47)
GSTM1			
Active	82 (43.6)	70 (46.1)	1
Null	106 (56.4)	82 (53.9)	1.19 (0.77–1.85)
GSTT1			
Active	151 (80.3)	122 (80.3)	1
Null	37 (19.7)	30 (19.7)	0.96 (0.56–1.66)
GST combined			
Active (at least one)	161 (85.6)	135 (88.8)	1
Null (both)	27 (14.4)	17 (11.2)	1.30 (0.67–2.50)
GST combined/skin photo-type			
Active/III–IV	48 (25.5)	71 (47.0)	1
Active/I–II	113 (60.1)	63 (41.7)	2.74 (1.67–4.51)
Null/III–IV	14 (7.5)	11 (7.3)	1.94 (0.80–4.70)
Null/I–II	13 (6.9)	6 (4.0)	3.06 (1.07–8.77)

^aTotals may vary because of missing value.

^bOdds ratio adjusted for age and sex.

^cI: always burns, never tans; II: often burns, tans minimally; III: rarely burns, tans well; IV: never burns, tans profusely.

NMSC; non-melanoma skin cancer; OR: odds ratio; CI: confidence intervals.

conducted separate analysis for subjects exposed and non-exposed to sunburns (Table II). An increased risk was suggested for subjects exposed to sunburns (OR 2.77; 95% CI 0.56–13.8) in comparison with non-exposed (OR 1.24; 95% CI 0.44–3.53).

Table II. Association between GSTM1, GSTT1 polymorphisms and cutaneous melanoma: multivariate analysis

	All subjects (n=340) OR (95% CI) ^a	Subjects exposed to sunburns (n=180) OR (95% CI) ^b	Subjects non-exposed to sunburns (n=111) OR (95% CI) ^b
GSTM1			
Active	1	1	1
Null	1.06 (0.63–1.78)	2.68 (0.93–7.74)	0.81 (0.40–1.63)
GSTT1			
Active	1	1	1
Null	1.28 (0.66–2.47)	1.37 (0.35–5.36)	1.08 (0.46–2.53)
GST combined			
Active (at least one)	1	1	1
Null (both)	1.82 (0.83–4.01)	2.77 (0.56–13.8)	1.24 (0.44–3.53)
GST combined/skin photo-type			
Active/III–IV	1	1	1
Active/I–II	2.01 (1.10–3.68)	3.33 (1.01–11.0)	1.55 (0.70–3.42)
Null/III–IV	2.85 (1.00–8.14)	10.0 (0.73–137.8)	1.54 (0.43–5.53)
Null/I–II	2.05 (0.62–6.74)	3.57 (0.47–27.3)	1.27 (0.21–7.48)

^aOdds ratio adjusted for age, sex, education, hair colour, skin photo-type, common naevi, solar lentigines and sunburns episodes in childhood.

^bOdds ratio adjusted for age, sex, education, hair colour, skin photo-type, common naevi and solar lentigines.

OR: odds ratio; CI: confidence intervals.

As an interaction was also suggested between GST null group and skin photo-type (OR 0.36, 95% CI 0.07–1.76) we combined skin photo-type and GST in one variable, using as a referent category (at least on active variant) and photo-type III/IV (burns minimally and tans easily) and re-ran the analysis. Subjects belonging to the GST null group and with photo-type I/II have very similar risk estimates (OR 2.05; 95% CI 0.62–6.74) to subjects belonging to the GST active group (at least one active variant) (OR 2.01; 95% CI 1.10–3.68). Subjects belonging to the GST null group and with photo-type III/IV (OR 2.85; 95% CI 1.00–8.14) were at a higher risk of melanoma than subjects with at least one active variant and photo-type III/IV. The OR increased to 10 (95% CI 0.73–137.8), among the sub-group of subjects exposed to sunburns and with GST null group and with photo-type III/IV although these were associated with wide confidence intervals (Table II).

Among the group with both null variants, subjects reporting sunburns in childhood were at an increased risk for melanoma (OR 9.16; 95% CI 1.18–70.9) in comparison with subjects with no sunburns (Table III).

Table SII (available at <http://www.medicaljournals.se/acta/content/?doi=10.2340/00015555-1078>) shows a

description of characteristics of the subjects with both null variants and the subjects with at least one active variant. In our study subjects with both null variants were mainly skin photo-type III/IV.

DISCUSSION

This is the first study to investigate the possible interaction between sun exposure and GSTM1 and GSTT1 polymorphisms in determining cutaneous melanoma risk. We observed a novel interaction between GST null polymorphisms and sunburns in childhood. Our findings suggest that the homozygous deletions in the genes encoding GSTM1 and GSTT1, among subjects with history of sunburns in childhood, a known risk factor for melanoma (2), further act to elevate melanoma risk. Subjects carrying both GSTM1 null and GSTT1 null genotype and having sunburns had a nine-fold increased risk of melanoma. The null polymorphism in both GSTM1 and GSTT1 genes results in lack of GSTM1 and GSTT1 proteins and the consequence is null conjugation activity (17). GSTM1 and GSTT1 are enzymes that detoxify products of oxidative stress caused by UV radiation. GSTM1 and GSTT1 detoxify

Table III. Association between sun exposure and cutaneous melanoma by glutathione S-transferase (GST) polymorphisms

	GST combined null (both)			GST combined active (at least one)		
	Cases (n=27) ^a n (%)	Controls (n=17) ^a n (%)	OR (95% CI) ^b	Cases (n=161) ^a n (%)	Controls (n=135) ^a n (%)	OR (95% CI) ^b
Sunburns in childhood						
No	10 (43.5)	13 (81.3)	1	67 (50.0)	90 (76.3)	1
Yes	13 (56.5)	3 (18.7)	9.16 (1.18–70.9)	67 (50.0)	28 (23.7)	3.11 (1.79–5.43)
Lifetime sun exposure (h)						
Low (≤26)	12 (54.6)	4 (23.5)	1	58 (38.7)	41 (31.5)	1
Medium (27–36)	3 (13.6)	8 (47.1)	0.07 (0.01–0.62)	47 (31.3)	53 (40.8)	0.69 (0.39–1.25)
High (≥37)	7 (31.8)	5 (29.4)	0.45 (0.07–3.23)	45 (30.0)	36 (27.7)	1.12 (0.58–2.19)

^aTotals may vary because of missing value.

^bOdds ratio adjusted for age, sex and education.

a variety of electrophilic compounds, oxidized lipid, and DNA products generated by reactive oxygen species-induced damage to intracellular molecules (18, 19). Therefore, by affecting the individual's ability to detoxify oxidative stress-related products, GSTM1 and GSTT1 null polymorphisms may influence the severity of the cutaneous damage. A recent study showed that GSTM1 null melanoma patients with a history of sunburn have increased levels of both DNA fragmentation evaluated by comet assays and mitochondrial DNA deletions in comparison with melanoma patients with no history of sunburn (20). Consistently with these findings our data suggest that exposure to solar radiation early in life, among GSTM1 and GSTT1 null genotypes increase melanoma risk through long-term cellular changes.

Kanestky et al. (14) showed that, among individuals with red or blond hair, those with cutaneous melanoma were twice as likely to carry GSTM1 and GSTT1 null genotypes compared with those without cutaneous melanoma. Our findings are in agreement with the study of Mössner et al. (12), which found no increased risk of melanoma among subjects with GSTM1 and GSTT1 null genotypes in the sub-group of people with red or blond hair. As an interaction was also suggested between GST null group and skin photo-type, we combined both variables and re-ran the analysis using as referent group the photo-type III/IV with at least one active variant. Epidemiological studies suggested that subjects with skin photo-type III/IV are more protected from sun damage than subjects with skin photo-type I/II (21). However, our findings suggest that if subjects are skin photo-type III/IV, but are carriers of both GSTM1 and GSTT1 null variants, the risk of melanoma can be as high as if they were skin photo-type I/II.

Kerb et al. (22) demonstrated that subjects carrying GSTT1 null genotype have lower minimal erythema doses (MED) in comparison with those who expressed GSTT1 protein. MED have been suggested to increase melanoma risk independently of skin colour. Subjects with low MED had twice the risk of melanoma compared with subjects with high MED. Further support for the importance of these genes in the protection of the skin against UV come from studies in patient with systemic lupus erythematosus (23, 24). Ollier et al. (23) showed that GSTM1 null individuals have an increased production of anti-Ro antibodies, a phenotype associated with marked photosensitivity. Fraser et al. (24) showed that subjects with lupus erythematosus and GSTM1 null genotype were more susceptible to the effects of sun exposure.

In the present study neither GSTM1 nor GSTT1 genes were independent risk factors for melanoma, which is in agreement with the results of a previous study (12). However, the results of our study support the findings that subjects carrying both GSTM1 and GSTT1 "null"

genotypes are at an increased risk of UVR cutaneous damage (25) and reinforce the hypothesis of the role of GST in detoxifying reactive oxygen species (ROS) produced by UV radiation during melanomagenesis. Moreover, this study confirms the hypotheses that individuals with multiple null genotypes at loci encoding detoxification enzymes have an increased risk of cancer (9).

Misclassification of sunburns history could be a limitation of our study. Part of this misclassification is a result of sun exposure questionnaire being an imperfect measure of sun exposure history. In order to overcome the problem we validated our questionnaire using two independent measures as suggested elsewhere (26). It has been suggested that total sun exposure predicts dermal elastosis and sunburns and intermittent sun exposure predict number of naevi (27–30). We compare sun exposure variables assessed by the questionnaire and skin damage variables assessed by a dermatologist following the International Agency for Research on Cancer (IARC) protocol (15). Overall, there was a high association between the two measurements. For example, we found that total sun exposure and occupation sun exposure were highly associated with dermal elastosis ($p < 0.0001$) and sunburns in childhood with number of naevi in adults ($p < 0.0001$) and time spent in the sun during holidays in childhood and number of naevi ($p = 0.003$). Moreover, we evaluated the reproducibility between answers in two different periods (7 years apart) and we found an agreement for sunburns in childhood of 80% (Cohen's kappa: 0.58).

Further research effort should focus on clarifying the link between the GSTM1 and GSTT1 "null" genotypes and increased susceptibility to sun exposure in early life. Finally, it seems important to understand which individuals are more at risk, how that risk can be modified and how the knowledge can be used for targeted surveillance.

ACKNOWLEDGEMENTS

This work was supported by the Istituto Dermatologico dell'Immacolata, Istituto di Ricovero a Carattere Scientifico (Progetto Ricerca Corrente 2008, Italian Ministry of Health) and by the Consulenza Tecnica Accertamento Rischi e Prevenzione – Istituto Nazionale per l'Assicurazione contro gli Infortuni sul lavoro (INAIL). We are indebted to Cristina Emanuel, from L'Oreal for providing a skin and a hair colour chart, which helped us to determine skin and hair colour during the interview, and to Valeria Fano for helpful comments on the analysis.

The authors declare no conflicts of interest.

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