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Polymorphisms in genes involved in DNA repair, cell growth, oxidative stress, and inflammatory response and melanoma risk

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Few genetic risk factors for melanoma have been examined and confirmed. We examined five important candidate genes (*MGMT*, *ICAM-5*, *VEGF*, *MPO*, and *TNF*-α) involved in direct reversal DNA repair, cell growth, oxidative stress, and inflammatory response, in relation to melanoma susceptibility. Eight candidate SNPs in the five genes were selected according to previous studies (*MGMT* 84Leu>Phe (rs12917), *MGMT* 143Ile>Val (rs2308321) [1], *VEGF* 398G>C (rs2010963), *VEGF* 1967C>T (rs3025039) [2], *MPO* −764T>C (rs2243828) [3], *TNF*-α−487A>G (rs1800629) [4] and *ICAM5* 301Val>Ile (rs1056538), *ICAM5* −542C>G (rs281439) [5]). Due to genotyping difficulty, the *MPO* −463G>A (rs2333227) was replaced by the MPO −764 T>C (rs2243828). Genotyping concordance between the two SNPs was 100%. (http://snp500cancer.nci.nih.gov). We investigated the associations between these genetic variants and melanoma susceptibility in a nested case-control study of 219 incident melanoma cases and 219 matched controls within the Nurses' Health Study (NHS) Cohort. The detailed information about the study population and laboratory assays were described elsewhere [6].

The detailed statistical analyses have been published previously [6]. We used unconditional logistic regression to evaluate the association between genotype and melanoma risk, firstly adjusting for matching factors (age and race (Caucasians and missing)), and then additionally adjusting for other melanoma risk factors. We evaluated the interactions between SNPs and melanoma risk factors (constitutional susceptibility score and cumulative sun exposure with a bathing suit) on melanoma susceptibility. We used the expectation maximization algorithm to estimate haplotype frequencies in cases and controls for the SNPs MGMT 84Leu>Phe and 143Ile>Val. We also explored the potential modification by antioxidants intake on the

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association between the MPO polymorphism and melanoma risk (See previous report for data collection and analysis [7]).

No departure from the Hardy-Weinberg Equilibrium among controls was observed for all the 8 SNPs. We observed that the *MGMT* 84Leu>Phe polymorphism was associated with melanoma susceptibility. The Phe carriers had a higher risk of melanoma than non-carriers (84Leu/Leu) with OR (95%CI) 1.55 (0.99–2.44). The association was attenuated in the multivariate model. The *MGMT* gene encodes a direct reversal DNA repair protein that removes alkyl or methyl adducts from the O^6 position of guanine to an internal cysteine residue at codon 145 of the protein [2]. An animal study suggested that MGMT protein prevents N-nitroso-N-methylurea-initiated skin carcinogenesis through avoiding tumor initiation triggered by alkylation damage [8]. There are three common non-synonymous polymorphisms in the *MGMT* gene, 84Leu>Phe, 143Ile>Val, and 178 Lys>Arg, and the latter two are in linkage disequilibrium. The Leu84 and Ile143 are close to the reactive cysteine in the 3D structure and may have subtle functional consequences [1]. The minor alleles in the 84Leu>Phe and 143Ile>Val polymorphisms were reported to be inversely associated with the capacity to repair tobacco-induced DNA damage [9]. This evidence suggests that these two minor alleles may confer a higher cancer susceptibility. Consistently, we observed a higher risk of melanoma for the 84Phe carriers and the 143Val carriers compared to their wildtypes. The 84Phe carriers had about a 50% increased risk of melanoma compared to non-carriers (84Leu/Leu). The 143Val carriers had an OR of 1.34(0.86–2.09) for melanoma risk compared to non-carriers (143Ile/Ile). Comparing to the common haplotype 84Leu/143Ile, the haplotype 84Phe/143Ile and 84Leu/143Val had an OR (95%CI) of 1.75 (1.11–2.76) and 2.00 (1.21–3.29), respectively. These associations remained significant after adjusting for other risk factors. Our results combined with the functional results of these two SNPs suggest that alkylation-related DNA damage (or other damage repaired by MGMT) may be involved in melanoma carcinogenesis.

Because the ICAM expression in melanocytes was shown to be inhibited by alpha-melanocyte-stimulating hormone (α-MSH), a sun exposure–induced ligand for skin pigmentation [10], we evaluated the interactions between the *ICAM5* variants and the constitutional susceptibility score and cumulative sun exposure with a bathing suit on melanoma risk. Comparing to the first tertiles, the ORs (95%CIs) of the third tertiles were 3.25(1.44–7.36) for constitutional susceptibility score and 3.03(1.71–5.39) for cumulative sun exposure with a bathing suit. In the interaction analysis, the highest risk of melanoma was observed among women with the *ICAM5* 301 Val/Val genotype and highest susceptibility (OR, 5.73; 95%CI, 1.62–20.31) or greatest sun exposure (OR, 4.83; 95%CI, 1.79–13.06). The interactions did not approach statistical significance (Table 2).

We did not observe significant results for SNPs in other genes (Table 1), or the interaction between the MPO polymorphism and dietary intake of antioxidants on melanoma risk.

In conclusion, this is the first study of the *MGMT, ICAM5,* and *MPO* genetic polymorphisms in relation to melanoma susceptibility. We found a borderline-significant association between the *MGMT*84 Leu>Phe and melanoma risk, and significant associations between the MGMT haplotypes and melanoma risk. A higher risk of melanoma was observed among 84Phe or 143Val carriers who have lower alkylation-damage-repair capacity. The statistical power of our study to detect a modest association is limited, and larger studies are warranted to confirm these findings.

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Table 1
Polymorphisms in selected candidate genes and melanoma risk

Polymorphism	Cases (%)	Controls (%)	OR (95%CI)[1]	OR (95%CI)[2]
MGMT 84 Leu>Phe (rs12917)				
Wt	152 (71.0)	168 (79.2)	1.00	1.00
Het	60 (28.0)	43 (20.3)	1.55 (0.99, 2.44)	1.44 (0.87, 2.37)
Var	2 (0.9)	1 (0.5)		
P value			0.06	0.16
MGMT 143 Ile>Val (rs2308321)				
Wt	154 (72.0)	164 (77.4)	1.00	1.00
Het	54 (25.2)	46 (21.7)	1.34 (0.86, 2.09)	1.25 (0.76, 2.05)
Var	6 (2.8)	2 (0.9)		
P value			0.19	0.39
ICAM5 301 Val>Ile (rs1056538)				
Wt	85 (39.9)	68 (32.7)	1.00	1.00
Het	97 (45.5)	108 (51.9)	0.70 (0.46, 1.08)	0.78 (0.49, 1.26)
Var	31 (14.6)	32 (15.4)	0.75 (0.42, 1.36)	0.80 (0.41, 1.55)
P value			0.25	0.58
ICAM5 −542 C>G (rs281439)				
Wt	126 (59.4)	130 (61.6)	1.00	1.00
Het	71 (33.5)	76 (36.0)	1.10 (0.75, 1.63)	1.04 (0.68, 1.62)
Var	15 (7.1)	5 (2.4)		
P value			0.63	0.85
VEGF 398 G>C (rs2010963)				
Wt	88 (42.3)	93 (46.7)	1.00	1.00
Het	100 (48.1)	85 (42.7)	1.25 (0.83, 1.89)	1.14 (0.72, 1.81)
Var	20 (9.6)	21 (10.6)	1.02 (0.51, 2.02)	1.38 (0.64, 2.96)
P value			0.55	0.68
VEGF 1967 C>T (rs3025039)				
Wt	157 (75.1)	163 (79.9)	1.00	1.00
Het	49 (23.4)	39 (19.1)	1.35 (0.84, 2.15)	1.47 (0.86, 2.49)
Var	3 (1.4)	2 (1.0)		
P value			0.21	0.16
MPO −764 T>C (rs2243828)				
Wt	131 (63.0)	112 (56.9)	1.00	1.00
Het	68 (32.7)	76 (38.6)	0.78 (0.51, 1.19)	0.90 (0.57, 1.44)
Var	9 (4.3)	9 (4.6)	0.85 (0.32, 2.26)	0.87 (0.29, 2.65)
P value			0.50	0.89
TNF-α −487 A>G (rs1800629)				
Wt	156 (73.6)	140 (66.4)	1.00	1.00
Het	46 (21.7)	61 (28.9)	0.67 (0.43, 1.05)	0.63 (0.38, 1.04)
Var	10 (4.7)	10 (4.7)	0.89 (0.36, 2.22)	0.91 (0.33, 2.48)
P value			0.21	0.20

Wt: wild type; Het: heterozygote; Var: homozygous variant.

The number of participants does not sum to total women because of missing data on genotype.

Some percentages among cases or controls do not sum to 100% due to rounding.

The p values were calculated based upon 2-degree-of-freedom (df) test for ICAM5 301 Val>Ile, VEGF 398 G>C, TNF-α −487 A>G, and MPO −764 T>C; 1 df test for the other SNPs.

[1] Unconditional logistic regression adjusted for the matching variables (age and race (Caucasians and missing)).

[2] Unconditional logistic regression adjusted for matching variables, constitutional susceptibility score, family history of melanoma, number of lifetime severe sunburns that blistered (0, 1–5, 6–11, or >11), sunlamp use or tanning salon attendance (yes/no), cumulative sun exposure while wearing a bathing suit, and geographic region.

Table 2

Interaction between ICAM5 301 Val>Ile and risk factors on melanoma risk

	Val/Val		Val/Ile		Ile/Ile	
	Cases/Controls	**OR (95%CI)**	**Cases/Controls**	**OR (95%CI)**	**Cases/Controls**	**OR (95%CI)**
Constitutional susceptibility score [1]						
Low	15/12	1.00	21/28	0.83 (0.29, 2.35)	6/9	1.02 (0.25, 4.23)
Medium	13/35	0.73 (0.20, 2.68)	26/34	2.15 (0.60, 7.67)	5/12	0.92 (0.19, 4.50)
High	57/21	5.73 (1.62, 20.31)	50/46	1.95 (0.58, 6.63)	20/11	3.10 (0.76, 12.62)
p for interaction = 0.12						
Cumulative sun exposure with a bathing suit [2]						
Low	14/24	1.00	16/33	1.00 (0.38, 2.66)	7/9	0.98 (0.26, 3.65)
Medium	17/18	1.57 (0.57, 4.34)	22/33	1.45 (0.57, 3.68)	8/10	1.96 (0.55, 6.94)
High	38/13	4.83 (1.79, 13.06)	41/29	2.30 (0.94, 5.64)	13/9	2.59 (0.76, 8.80)
p for interaction = 0.34						

[1] Unconditional logistic regression adjusted for the matching variables, family history of melanoma, number of lifetime severe sunburns, sunlamp use or tanning salon attendance, cumulative sun exposure while wearing a bathing suit, and geographic region.

[2] Unconditional logistic regression adjusted for the matching variables, constitutional susceptibility score, family history of melanoma, number of lifetime severe sunburns, sunlamp use or tanning salon attendance, and geographic region.