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Genetic Variants in the *53BP1* Gene and Skin Cancer Risk

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TO THE EDITOR

The conserved p53-binding protein 1 (53BP1) was initially identified as a nuclear protein that interacts with the DNA-binding domain of tumor suppressor p53 and enhances p53-mediated transcription activation (Iwabuchi *et al.*, 1998). The interaction region of 53BP1, BRCT (BRCA1 C-terminus) repeats, are present in several proteins involved in DNA repair and cell cycle control, suggesting a direct role of 53BP1 in the cellular response to DNA damage and maintenance of genomic stability (Rappold *et al.*, 2001; Joo *et al.*, 2002). Recently, studies suggested that 53BP1 is a central mediator of the DNA damage checkpoint signaling (Wang *et al.*, 2002) and it directly participates in the repair for DNA double-strand breaks (Huyen *et al.*, 2004). 53BP1-deficient mice exhibit growth retardation, high-radiation sensitivity, and tumor development features that are indicative of a defective DNA damage response (Ward *et al.*, 2003). 53BP1 has been shown to constitutively have an important role in the etiology of human cancer (DiTullio *et al.*, 2002). It is plausible that sequence variation in the regulatory and coding regions of the *53BP1* gene might affect its transcription and protein structure thus, its biological function in checkpoint signaling and DNA repair, leads to susceptibility to cancers.

In this study, we hypothesized that that common genetic variants of *53BP1* were associated with risk of skin cancer. We comprehensively surveyed common genetic variation of the *53BP1* gene using two complementary approaches, including putatively functional single nucleotide polymorphisms (SNPs) and choosing tagging-SNPs in the *53BP1* gene locus (introns and exons as well as 20 kb upstream and 20 kb downstream of the coding region). We performed a skin cancer case-control study of Caucasians nested within the Nurses' Health Study to evaluate whether these common genetic variants are associated with the risks of non-melanoma skin cancers (squamous cell carcinoma (SCC) and basal cell carcinoma (BCC)) along with melanoma risk. We further investigated the association of these genetic variants with pigmentary phenotypes (hair color, skin color, tanning ability, and the number of moles). The nested case-control study consisted of 218 incident melanoma cases, 285 incident SCC cases, 300 incident BCC cases, and 870 age-matched controls. A detailed description of the selection and characteristics of cases and controls was published previously (Han *et al.*, 2005).

We included four putatively functional SNPs that have been identified in the promoter and coding regions of *53BP1* (http://egp.gs.washington.edu): rs1869258, rs560191, rs689647, and rs2602141. Using genotype data from the 90 CEU samples in the HapMap project, we further selected four tagging-SNPs by the Tagger program ($r^2 > 0.8$): rs3862138, rs17782975, rs2242069, and rs999047 (Supplementary Figure S1 online and Supplementary Table S1 online). We genotyped the eight SNPs by the 5′ nuclease assay (TaqMan) in 384-well format, using the ABI PRISM 7900HT Sequence Detection System (Applied Biosystems, Foster City, CA). The distributions of genotypes for the eight SNPs were in Hardy–Weinberg equilibrium among controls. Because the three SNPs (rs1869258, rs560191, and rs2602141) are in high linkage disequilibrium (each pair-wise $r^2 > 0.9$, Supplementary Table S2 online), we anticipated that these three SNPs would show similar effects on pigmentary phenotypes and skin cancer risk.

We evaluated the main effect of the selected polymorphisms across the three types of skin cancer using unconditional logistic regression (Table 1). In the analyses controlling for the age, we found that three SNPs (rs689647, rs2242069, and rs999047) were consistently associated with a significantly decreased risk of BCC (additive odds ratio and 95% confidence interval: 0.69 (0.50–0.95), 0.68 (0.51–0.90), and 0.66 (0.48–0.91), respectively). These three SNPs are in moderate linkage disequilibrium (pair-wise r^2 between 0.27 and 0.70, Supplementary Table S2 online).

Abbreviations: BCC, basal cell carcinoma; SCC, squamous cell carcinoma; SNP, single nucleotide polymorphism

Table 1. Associations between selected SNPs in *53BP1* region and skin cancer risk

		Melanoma		SCC		BCC	
	Controls (%)	Cases (%)	Multivariate OR[1]	Cases (%)	Multivariate OR[1]	Cases (%)	Multivariate OR[1]
5′ UTR							
rs3862138							
CC	503 (62.9)	132 (63.5)	1.00 (ref)	173 (66.3)	1.00 (ref)	179 (65.1)	1.00 (ref)
CT+TT	297 (37.1)	76 (36.5)	0.99 (0.72–1.36)	88 (33.7)	0.85 (0.63–1.14)	96 (34.9)	0.90 (0.68–1.21)
Per copy of T			0.93 (0.71–1.24)		0.95 (0.74–1.21)		0.98 (0.77–1.24)
P for trend			0.64		0.66		0.86
Coding region							
rs17782975							
TT	670 (81.8)	168 (82.4)	1.00 (ref)	222 (81.0)	1.00 (ref)	227 (82.8)	1.00 (ref)
TC+CC	149 (18.2)	36 (17.6)	0.96 (0.64–1.44)	52 (19.0)	1.05 (0.74–1.49)	47 (17.2)	0.92 (0.64–1.33)
Per copy of C			1.00 (0.68–1.48)		1.10 (0.79–1.52)		0.92 (0.65–1.30)
P for trend			0.99		0.58		0.62
rs689647 (G412S)							
CC	635 (76.5)	155 (75.2)	1.00 (ref)	225 (82.1)	1.00 (ref)	243 (82.9)	1.00 (ref)
CT+TT	195 (23.5)	51 (24.8)	1.08 (0.76–1.55)	49 (17.9)	0.71 (0.50–1.01)	50 (17.1)	0.68 (0.48–0.95)
Per copy of T			1.05 (0.76–1.46)		0.74 (0.53–1.02)		0.69 (0.50–0.95)
P for trend			0.77		0.07		0.02
rs2602141 (K1136Q)[2]							
TT	389 (46.4)	86 (41.0)	1.00 (ref)	143 (52.4)	1.00 (ref)	154 (52.0)	1.00 (ref)
TG+GG	449 (53.6)	124 (59.0)	1.27 (0.93–1.72)	130 (47.6)	0.78 (0.60–1.03)	142 (48.0)	0.80 (0.61–1.05)
Per copy of G			1.05 (0.83–1.34)		0.85 (0.69–1.06)		0.86 (0.70–1.06)
P for trend			0.67		0.14		0.15
rs2242069							
TT	545 (67.0)	132 (65.3)	1.00 (ref)	194 (72.9)	1.00 (ref)	212 (76.3)	1.00 (ref)
TG+GG	269 (33.0)	70 (34.7)	1.09 (0.79–1.52)	72 (27.1)	0.75 (0.55–1.02)	66 (23.7)	0.63 (0.46–0.86)
Per copy of *G*			1.13 (0.85–1.50)		0.83 (0.63–1.09)		0.68 (0.51–0.90)
P for trend			0.41		0.18		0.01
3′ UTR							
rs999047							
AA	597 (74.5)	136 (69.7)	1.00 (ref)	220 (80.3)	1.00 (ref)	226 (81.9)	1.00 (ref)
AG+GG	204 (25.5)	59 (30.3)	1.29 (0.91–1.82)	54 (19.7)	0.72 (0.51–1.00)	50 (18.1)	0.65 (0.46–0.91)
Per copy of *G*			1.23 (0.90–1.68)		0.82 (0.60–1.10)		0.66 (0.48–0.91)
P for trend			0.2		0.18		0.01
Haplotype (A B C D)[3]							
0 0 0 0	863 (61.1)	203 (59.3)	1.00	288 (61.5)	1.00	326 (65.3)	1.00
0 1 0 0	279 (19.8)	63 (18.5)	0.97 (0.70–1.35)	92 (19.7)	0.99 (0.75–1.30)	98 (19.5)	0.92 (0.71–1.21)
1 1 1 1	160 (11.3)	40 (11.7)	1.08 (0.73–1.60)	42 (9.0)	0.78 (0.54–1.13)	40 (8.0)	0.66 (0.45–0.96)
0 0 1 0	73 (5.2)	21 (6.2)	1.28 (0.76–2.17)	27 (5.7)	1.12 (0.69–1.82)	26 (5.2)	0.89 (0.55–1.43)
0 0 0 1	24 (1.7)	11 (3.2)	1.97 (0.96–4.07)	11 (2.4)	1.40 (0.68–2.87)	5 (1.1)	0.59 (0.22–1.61)
Rare<1% combined	12 (0.9)	4 (1.2)	1.42 (0.45–4.54)	8 (1.8)	1.92 (0.80–4.63)	4 (0.9)	0.89 (0.29–2.75)
P for global test			0.52		0.40		0.31

Abbreviations: BCC, basal cell carcinoma; OR, odds ratio; SCC, squamous cell carcinoma; SNP, single nucleotide polymorphism; UTR, untranslated region; *53BP1*, p53-binding protein 1.
[1]Unconditional logistic regression adjusted for age;
[2]The two SNPs (rs1869258 and rs560191) that are in high linkage disequilibrium with rs2602141 (each pair-wise $r^2 > 0.9$) showed similar associations with the risk of melanoma, SCC and BCC.
[3]Haplotype based on the four SNPs A: rs689647; B: rs2602141; C:rs2242069; D: rs999047; 0, wild-type allele; 1, variant allele.

Table 2. Associations between selected SNPs in *53BP1* region and hair color and moles among controls

	Hair color			Moles		
SNP	β[1]	SE	*P*-value	β[1]	SE	*P*-value
rs3862138	0.14	0.12	0.23	0.20	0.12	0.11
rs17782975	−0.01	0.16	0.96	0.45	0.18	0.01
rs689647	0.22	0.15	0.13	0.19	0.15	0.22
rs2602141[2]	0.22	0.10	0.03	0.31	0.11	0.003
rs2242069	0.21	0.13	0.10	0.10	0.13	0.44
rs999047	0.20	0.14	0.16	−0.08	0.14	0.57

Abbreviations: SNP, single nucleotide polymorphism; 53BP1, p53-binding protein 1.
[1]The regression parameter β refers to the mean change in scoring in hair color (black to blonde) and number of moles per copy of the SNP minor allele. We regressed an ordinal coding for hair color (1=black; 2=dark brown; 3=light brown; 4=blonde; and 5=red) or number of moles (1: none; 2: 1–2; 3: 3−5; 4: 6−9; 5: 10−14 and 5: 15+) on an ordinal coding for genotype (0, 1, or 2 copies of SNP minor allele). $\beta > 0$ means that the SNP is associated with lighter hair color and more moles.
[2]The two SNPs (rs1869258 and rs560191) that are in high linkage disequilibrium with rs2602141 (each pair-wise $r^2 > 0.9$) showed similar associations with hair color (*P*-value, 0.04 and 0.07, respectively) and moles (*P*-value, 0.02 and 0.003, respectively).

The non-synonymous SNP, rs689647 (G412S), has been investigated in a case-control study of breast cancer but found no association with breast cancer risk (Frank *et al.*, 2005). We did not observe significant association between three putatively functional SNPs (rs1869258, rs560191, and rs2602141) and any type of skin cancer, which was consistent with previous studies (Frank *et al.*, 2005; Ma *et al.*, 2006; Rapakko *et al.*, 2007). We further evaluated haplotypes based on the four SNPs (rs689647, rs2602141, rs2242069, and rs999047) that modified the risk of the cancer subtypes (Table 1). We found that the variant allele of rs689647 was often inherited together with that of the other three SNPs and formed a haplotype in 11% of the population. This haplotype was significantly associated with a decreased risk of BCC (odds ratio, 0.66; 95% confidence interval, 0.45–0.96), suggesting a protective effect on BCC compared with the most frequent haplotype carrying all wild-type alleles in approximately 60% of the population. However, the haplotype carrying only the variant allele of rs2242066 or rs999047 was not significantly associated with BCC risk as suggested in single-SNP analysis, though the latter showed a non-significant decreased risk of BCC. Of note, the last haplotype carrying only the variant allele of rs999047 was associated with a non-significant increased risk of melanoma (odds ratio, 1.97; 95% confidence interval, 0.96–4.07), which was stronger than the association of single-SNP analysis (odds ratio, 1.23; 95% confidence interval, 0.90–1.68). These associations did not change substantially after additional adjustment for potential risk factors including pigmentary phenotypes, family history of skin cancer, geographic region, accumulative sun exposure, sunburns, and sunlamp use or tanning salon attendance. We did not observe any statistically significant interaction between pigmentary phenotypes and genetic variants on skin cancer risk.

We evaluated the associations between the selected SNPs and pigmentary phenotypes among controls using linear regression models (Table 2). We found the three highly correlated putatively functional SNPs (rs1869258, rs560191, and rs2602141) were associated with lighter hair color (*P* for trend, 0.04, 0.07, and 0.03, respectively) and more moles (*P* for trend, 0.02, 0.003, and 0.003, respectively). We also found one intronic SNP in 5′ terminus, rs17782975, was significantly associated with more moles (*P* for trend, 0.01). No statistically significant associations between genetic variants and skin color or tanning ability were observed. To our knowledge, this is the first report that *53BP1* is associated with pigmentary phenotypes in Caucasians, although this gene has shown clear evidence of adaptive skin pigmentation selection in Africans (Izagirre *et al.*, 2006). Two other genes involved in DNA repair, *p53* and *MDM2*, have been reported to be associated with pigmentary phenotypes (Cui *et al.*, 2007; Nan *et al.*, 2009).

In summary, we systematically evaluated the common genetic variants of the *53BP1* gene and the risk of skin cancer. We found three SNPs (rs689647, rs2242069, and rs999047) were significantly associated with a decreased risk of BCC. These findings may lead to further replication and functional studies that will elucidate the underlying mechanisms of skin cancer development associated with these genetic variants.

CONFLICT OF INTEREST

The authors state no conflict of interest.

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SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at http://www.nature.com/jid

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No Evidence That Human Papillomavirus Is Responsible for the Aggressive Nature of Recessive Dystrophic Epidermolysis Bullosa–Associated Squamous Cell Carcinoma

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TO THE EDITOR

Recessive dystrophic epidermolysis bullosa (RDEB) is a devastating inherited skin disease caused by mutations in the gene encoding type VII collagen (Christiano *et al.*, 1993). The condition is characterized by skin fragility, trauma-induced skin blistering, and chronic non-healing wounds (Mellerio *et al.*, 2007). Patients with RDEB are at strongly increased risk of developing aggressive cutaneous squamous cell carcinoma (SCC), which is the cause of death by age 45 years in 70% of individuals with the most severe form of RDEB (Fine *et al.*, 2009). Potential similarities between the microenvironment of non-healing wounds and mucosal epithelia, wherein human papillomaviruses (HPVs) have been shown to induce the development of SCC (Zur Hausen, 2009), led us to investigate whether HPV infection could be responsible for the increased incidence and aggressive nature of RDEB SCC. The involvement of HPV in the development of cutaneous SCC remains controversial, except in the rare genodermatosis epidermodysplasia verruciformis, wherein up to 60% of patients develop SCC containing high copy numbers of beta-papillomavirus (β-PV) (Harwood and Proby, 2002), which may be restricted to a minority of tumor cells (Dell'Oste *et al.*, 2009). The possibility that HPV are also involved in RDEB SCC has not been addressed.

HPVs are a diverse group of small double-stranded DNA viruses that infect squamous epithelial cells. The two largest genera are alpha-papillomavirus (α-PV), comprising all HPV genotypes found in mucosal lesions as well as many of those associated with benign skin warts, and β-PV, containing HPV types, which have most frequently been associated with epidermodysplasia verruciformis and cutaneous SCC (de Villiers *et al.*, 2004). Because vaccines against prevalent high risk mucosal/genital HPV (HPV16/18) are available and effective at preventing cancer development (Zur Hausen, 2009), we tested RDEB SCC for the presence of 18 high risk α-PV types using the digene HPV genotyping reverse hybridization assay detection kit (Qiagen, Leiden, The Netherlands). We tested DNA prepared from 21 separate SCC isolated from 12 RDEB patients as well as DNA from 39 organ transplant recipient SCC and 18 immunocompetent SCC collected for a separate study (Purdie *et al.*, 2009) with 6 vulval SCC samples included as positive controls. All DNA were isolated from frozen tissue collected after informed consent and in accordance with Helsinki guidelines following research ethics committee approval. All cutaneous SCC from RDEB and non-RDEB individuals were negative, whereas nine different α-PV types were detected in the vulval SCC samples.

Next we tested the presence of β-PV using the reverse hybridization assay kit skin (β) HPV detection system (Diassay, Rijswijk, The Netherlands) (de Koning