View metadata, citation and similar papers at core.ac.uk
brought to you by CORE
provided by Elsevier - Publisher Connector

new dominant syndrome. I. Clinical and histological features. *Clin Genet* 15:228–38

Fitch KR, McGowan KA, van Raamsdonk CD, Fuchs H, Lee D, Puech A *et al.* (2003) Genetics of dark skin in mice. *Genes Dev* 17: 214–28

Gu LH, Kim SC, Ichiki Y, Park J, Nagai M, Kitajima Y (2003) A usual frameshift and delayed termination codon mutation in keratin 5 causes a novel type of epidermolysis bullosa simplex with migratory circinate erythema. *J Invest Dermatol* 121:482–5

Hamada T, Kawano Y, Szczecinska W, Wozniak K, Yasumoto S, Kowalewski C *et al.* (2005) Novel keratin 5 and 14 gene mutations in patients with epidermolysis bullosa simplex from Poland. *Arch Dermatol Res* 296:577–9

Hassing JH, Doeglas HM (1980) Dystrophia bullosa hereditaria, typus maculatus (Mendes da Costa–van der Valk): a rare genodermatosis. *Br J Dermatol* 102:474–6

Heimer WL II, Brauner G, James WD (1992) Dermatopathia pigmentosa reticularis: a report of a family demonstrating autosomal dominant inheritance. *J Am Acad Dermatol* 26(Part 2):298–301

Horigushi Y, Sawamura D, Mori R, Nakamura H, Takahashi K, Shimizu H (2005) Clinical heterogeneity of 1649delG mutation in the tail domain of keratin 5: a Japanese family with epidermolysis bullosa simplex with mottled pigmentation. *J Invest Dermatol* 125: 83–5

Huber M, Floeth M, Borradori L, Schacke H, Rugg EL, Lane EB *et al.* (2002) Deletion of the cytoplasmatic domain of BP180/collagen XVII causes a phenotype with predominant features of epidermolysis bullosa simplex. *J Invest Dermatol* 118:185–92

Irvine AD, Rugg EL, Lane EB, Hoare S, Peret C, Hughes AE *et al.* (2001) Molecular confirmation of the unique phenotype of epidermolysis bullosa simplex with mottled pigmentation. *Br J Dermatol* 144:40–5

Itin PH, Lautenschlager S, Meyer R, Mevorah B, Rufli T (1993) Natural history of the Naegeli–Franceschetti–Jadassohn syndrome and further delineation of its clinical manifestations. *J Am Acad Dermatol* 28:942–50

Jonkman MF, Pas HH, Nijenhuis M, Kloosterhuis G, Steege G (2002) Deletion of a cytoplasmic domain of integrin beta4 causes epidermolysis bullosa simplex. *J Invest Dermatol* 119: 1275–8

Lane EB, Rugg EL, Navsaria H, Leigh IM, Heagerty AH, Ishida-Yamamoto A *et al.* (1992) A mutation in the conserved helix termination peptide of keratin 5 in hereditary skin blistering. *Nature* 356:244–6

Lanschuetzer CM, Emberger M, Laimer M, Diem A, Bauer JW, Soyer HP *et al.* (2005) Epidermolysis bullosa naevi reveal a distinctive dermoscopic pattern. *Br J Dermatol* 153: 97–102

Mason PJ, Wilson DB, Bessler M (2005) Dyskeratosis congenita – a disease of dysfunctional telomere maintenance. *Curr Mol Med* 5:159–70

McLean WH (2003) Genetic disorders of palm skin and nail. *J Anat* 202:133–41

Petronius D, Bergman R, Ben Izhak O, Leiba R, Sprecher E (2003) A comparative study of immunohistochemistry and electron microscopy used in the diagnosis of epidermolysis bullosa. *Am J Dermatopathol* 25:198–203

Pfendner E, Rouan F, Uitto J (2005) Progress in epidermolysis bullosa: the phenotypic spectrum of plectin mutations. *Exp Dermatol* 14:241–9

Shemanko CS, Mellerio JE, Tidman MJ, Lane EB, Eady RA (1998) Severe palmo-plantar hyperkeratosis in Dowling–Meara epidermolysis bullosa simplex caused by a mutation in the keratin 14 gene (KRT14). *J Invest Dermatol* 111:893–5

Smith FJ, Eady RA, Leigh IM, McMillan JR, Rugg EL, Kelsell DP (1996) Plectin deficiency results in muscular dystrophy with epidermolysis bullosa. *Nat Genet* 13:450–7

Sprecher E, Yosipovitch G, Bergman R, Ciubutaro D, Indelman M, Pfendner E *et al.* (2003) Epidermolytic hyperkeratosis and epidermolysis bullosa simplex caused by frameshift mutations altering the V2 tail domains of keratin 1 and keratin 5. *J Invest Dermatol* 120:623–6

Uttam J, Hutton E, Coulombe PA, Anton-Lamprecht I, Yu QC, Gedde-Dahl T Jr *et al.* (1996) The genetic basis of epidermolysis bullosa simplex with mottled pigmentation. *Proc Natl Acad Sci USA* 93:9079–84

Uitto J, Richard G (2005) Progress in epidermolysis bullosa: from eponyms to molecular genetic classification. *Clin Dermatol* 23:33–40

The A148T Variant of the *CDKN2A* Gene Is Not Associated with Melanoma Risk in the French and Italian Populations

Journal of Investigative Dermatology (2006) **126,** 1658–1660. doi:10.1038/sj.jid.5700293; published online 13 April 2006

TO THE EDITOR

CDKN2A (OMIM 600160) is the major melanoma susceptibility gene identified to date, which predisposes to familial melanoma and multiple primary melanoma (MPM). Germline *CDKN2A* mutations have been detected with a frequency varying from 5 to 46% in melanoma families from different countries (Holland *et al.*, 1995; Walker *et al.*, 1995; FitzGerald *et al.*, 1996; Soufir *et al.*, 1998). Different ethnic and environmental factors might account for such different percentages. The majority of *CDKN2A* germline mutations has been found in exons 1α and 2 of the *CDKN2A* gene affecting predominantly the $p16^{INK4A}$ transcript (Stahl *et al.*, 2004). However, a specific role for the $p14^{ARF}$ transcript has been suggested by the identification of a germline deletion of the *CDKN2A* gene involving exon 1β (Hewitt *et al.*, 2002) and of five different germline mutations at the $p14^{ARF}$ exon 1β splice donor site in familial melanoma kindreds (Harland *et al.*, 2005).

Three polymorphisms have been detected in the *CDKN2A* gene including one coding variant (c.442G>A) localized in exon 2 and two noncoding variants (c.500C>G and c.540C>T) localized in the 3′ untranslated region.

The c.442G>A variant converts an alanine (GCG) to a threonine (ACG) residue at codon 148 (A148T), located in the fourth ankyrin repeat domain, and has no recognized effect on $p16^{INK4A}$ function (Ranade *et al.*, 1995; Lilischkis *et al.*, 1996). The A148T allele frequency has been reported to vary from 1.5% in the CEPH population (Hussussian *et al.*, 1994) to 2% in the Utah population (Kamb *et al.*, 1994), and it has been recently suggested to be a low penetrance melanoma susceptibility allele in a population from Poland (Debniak *et al.*, 2005).

Abbreviations: CI, confidence interval; MPM, multiple primary melanoma; OR, odds ratio

The c.500C>G and the c.540C>T polymorphisms were not shown to be overrepresented in melanoma patients (Aitken *et al.*, 1999).

To determine the contribution of the A148T variant on melanoma risk, we genotyped this allele in two European populations from France and Italy. An overall number of 500 French melanoma patients comprising 405 sporadic melanoma patients, 57 familial melanoma patients and 38 sporadic multiple primary melanoma patients were recruited from the MELAN-COHORT, a prospective melanoma cohort including all melanoma patients from Departments of Dermatology of different Hospitals in Paris (Bichat, Percy, Ambroise Paré, Henri Mondor, Cochin and Saint-Louis Hospitals). In addition, 119 Italian sporadic melanoma patients were enrolled at the Department of Dermatology of the University of L'Aquila, L'Aquila, Italy. The control groups, matched by sex and age (within ±1 year) to the case groups, were recruited among patients affected by diseases unrelated to melanoma attending the same Hospitals and were composed of 143 French and 121 Italian control subjects with no personal or family history of skin cancer. The number of common melanocytic nevi and the presence or absence of clinically atypical nevi and of atypical mole syndrome were recorded for all Italian melanoma patients, for 184 French melanoma patients and for all French and Italian controls through physical examination by a dermatologist. Subjects were classified as having ⩽50 or >50 common melanocytic nevi. A detailed medical and family history with regard to cutaneous and extracutaneous malignant neoplasms was also obtained. Histopathologic features of melanoma, tumor site, and age at diagnosis were also recorded.

After genomic DNA extraction from peripheral blood leukocytes using the QIAamp Blood Mini Kit (QIAgen GmbH, Hilden, Germany), the A148T allele was genotyped using the Taqman SNP Genotyping Assay–allelic discrimination method (Applied Biosystems, Foster City, CA). The study was approved by the Local Ethical Committees and The Declaration of Helsinki Principles were followed. Written informed consent was obtained from all participants.

The genotype frequency of the A148T variant in the melanoma and control groups was compared using χ^2 test and two-sided *P*-value. Odd ratios (OR) were also estimated by comparing at risk genotypes (homozygotes AA + heterozygotes AG) versus the ''reference or consensus'' genotype (homozygotes GG). In addition, in order to evaluate the risk according to different age groups, melanoma patients were divided into two different groups based on the age at diagnosis: ⩽50 years and >50 years. Genotypes and allele frequency of the A148T allele in the French and Italian populations are shown in Table 1. Genotype frequency in both melanoma groups and both control groups was in Hardy–Weinberg equilibrium. The frequency of the mutant allele A was 6.7% in the Italian melanoma patients and 6.2% in the corresponding control subjects (OR 1.10, 95% confidence interval (CI) (0.51–2.37); $P=0.80$), 3.4% in the French melanoma group and 4.2% in the French controls (OR 0.77, 95% CI (0.39–0.52); $P=0.46$) (Table 1). Three subjects were homozygotes for the mutant sequence, including two melanoma patients (one in the French melanoma group and one in the Italian melanoma group) and one Italian control subject. An overall significant statistical difference in the A148T allele frequency was detected between Italian and French subjects ($P=0.008$).

No association of the A148T allele with melanoma risk was detected in the group of patients ⩽50 years of age at diagnosis (French population: OR 1.53, 95% CI (0.41–1.55); $P=0.44$; Italian population: OR 0.38, 95% CI (0.11–1.27); $P=0.12$). The total number of common melanocytic nevi and the presence of clinically atypical nevi were not associated with the A148T allele in both populations. In addition, in the French cohort, the A148T was not associated with any melanoma subgroup, that is, sporadic melanoma (allele frequency 3.4%, $P=0.88$), familial melanoma (allele frequency 4.4%, $P=0.9$), and sporadic multiple primary melanoma (allele frequency 4%, $P=0.96$).

The role of the A148T allele in melanoma has been investigated in a few studies reporting discordant results in different series of melanoma patients (Aitken *et al.*, 1999; Ghiorzo *et al.*, 1999; Kumar *et al.*, 2001; Bertram *et al.*, 2002; Debniak *et al.*, 2005) (Table 2). Ghiorzo *et al.* (1999) identi-

Table 1. Frequency of the A148T allele in melanoma cases and controls of a French and an Italian population

No. of subjects	Genotype (%)	c.442 allele frequency	*P*-value	OR (95% CI)
119 Italian patients	A/A 1 (0.84) G/A 14 (11.76) G/G 104 (87.4)	6.72%	0.81	1.10 (0.51–2.37)
121 Italian controls	A/A 1 (0.83) G/A 13 (10.74) G/G 107 (88.43)	6.2%		
500 French patients	A/A 1 (0.2) G/A 32 (6.4) G/G 467 (93.4)	3.4%	0.46	0.77 (0.39–1.52)
143 French controls	A/A 0 (0.0) G/A 12 (8.4) G/G 131 (91.6)	4.2%		

CI, confidence interval; OR, odds ratio.

Table 2. Genotypes and allele frequencies of the A148T allele in different populations

Reference	Geographic origin	Study population	Genotype	Frequency
Ghiorzo *et al.* (1999)	North-Western Italy	Melanoma families (*n*=14)	NS[a]	5/14 (35.7%)
Bertram *et al.* (2002)	UK	AN/FM patients (AMS ⩾2 or familial melanoma or MPM patients) (*n*=488)	A/A	0/488 (0%)
			G/A	24/488 (4.9%)
			G/G	464/488 (95.1%)
		Population-based sample (*n*=599)	A/A	0/599 (0%)
			G/A	31/599 (5.2%)
			G/G	568/599 (94.8%)
Debniak *et al.* (2005)	Poland	Melanoma patients (*n*=471) (56 familial and 415 sporadic melanoma patients)	A/A	0/471 (0%)
			G/A	33/471 (7%)
			G/G	438/471 (93%)
		Controls (*n*=1,210)	A/A	0/1210 (0%)
			G/A	35/1210 (2.89%)
			G/G	1175/1210 (97.1%)

AMS, atypical mole syndrome; AN, atypical nevus; FM, familial melanoma; MPM, multiple primary melanoma; NS, not specified.
[a]The number of individuals is not specified.

fied the A148T polymorphism in five of 14 Italian melanoma family members (OR 1.9, 95% CI (0.4–5.6)) with no segregation of this variant with the melanoma phenotype. In a subsequent study, the A148T was detected in 4.9% of adults from 179 English families with the atypical nevus phenotype and/or a family history of melanoma and in 5.2% of controls (Bertram *et al.*, 2002). Both studies did not show evidence for the A148T variant as a melanoma susceptibility allele.

In a large cohort of Polish patients, the A148T allele has been shown to be associated with an increased risk of cutaneous melanoma being detected in 7% of melanoma patients *versus* 2.89% of the control subjects with an OR of 2.529 (95% CI 1.552–4.121; $P=0.0003$), especially in patients diagnosed under 50 years of age (OR 3.443, 95% CI (1.862–6.367); $P=0.0002$) (Debniak *et al.*, 2005). Harland *et al.* (2000) demonstrated a linkage disequilibrium of the A148T allele with the 493A>T *CDKN2A* promoter variant, known to affect gene expression, which might be involved in melanoma susceptibility.

In our study, no significant difference of the A148T allele frequency was observed between melanoma patients and controls in the French and Italian populations, although the allele frequency was significantly different between these populations.

In conclusion, our results showed that the A148T allele is not associated with the risk of cutaneous melanoma nor with the age at melanoma diagnosis in the French and Italian populations. Assessment of this allele in other populations should further help to clarify the role of the A148T variant in melanoma predisposition.

CONFLICT OF INTEREST

The authors state no conflict of interest.

Tania Spica[1], Marc Portela[2], Bénédicte Gérard[2], Federica Formicone[1], Vincent Descamps[3], Béatrice Crickx[3], Laurence Ollivaud[4], Alain Archimbaud[4], Nicolas Dupin[5], Pierre Wolkenstein[6], Dominique Vitoux[7], Céleste Lebbe[4], Philippe Saiag[8], Nicole Basset-Seguin[4], Maria Concetta Fargnoli[1], Bernard Grandchamp[2], Ketty Peris[1], Nadem Soufir[2] and MELAN-COHORT[9]

[1]Department of Dermatology, University of L'Aquila, L'Aquila, Italy; [2]Laboratoire de Biochimie Hormonale et Génétique, Hôpital Bichat-Claude Bernard, AP-HP, Faculté de Médecine Paris VII, IFR02, Paris, France; [3]Service de Dermatologie, Hôpital Bichat-Claude Bernard, AP-HP, Faculté de Médecine Paris VII, Paris, France; [4]Service de Dermatologie, Hôpital Saint-Louis, AP-HP, Faculté de Médecine Paris VII, Paris, France; [5]Service de Dermatologie, Hôpital Tarnier, AP-HP, Faculté de Médecine Paris V, Paris, France; [6]Service de Dermatologie, Hôpital Henri Mondor, AP-HP, Faculté de Médecine Paris XIII, Créteil, France; [7]Service de Biochimie, Hôpital Saint-Louis, AP-HP, Faculté de Médecine Paris VII, Paris, France; [8]Service de Dermatologie, Hôpital Ambroise Paré, AP-HP, Faculté de Médecine Paris-Ile de France Ouest, Boulogne Billancourt, Paris, France and [9]MELAN-COHORT is a prospective cohort of melanoma patients constituted by all the University-affiliated Departments of Dermatology of Paris area.
E-mail: peris@univaq.it

REFERENCES

Aitken J, Welch J, Duffy D, Milligan A, Green A, Martin N *et al.* (1999) CDKN2A variants in a population-based sample of Queensland families with melanoma. *J Natl Cancer Inst* 91:446–52

Bertram CG, Gaut RM, Barrett JH, Pinney E, Whitaker L, Turner F *et al.* (2002) An assessment of the CDKN2A variant Ala148Thr as nevus/melanoma susceptibility allele. *J Invest Dermatol* 119:961–5

Debniak T, Scott RJ, Huzarski T, Byrski T, Rozmiarek A, Debniak B *et al.* (2005) CDKN2A common variants and their association with melanoma risk: a population based study. *Cancer Res* 65:835–9

FitzGerald MG, Harkin DP, Silva-Arrieta S, MacDonald DJ, Lucchina LC, Unsal H *et al.* (1996) Prevalence of germ-line mutations in p16, p19ARF, and CDK4 in familial melanoma: analysis of a clinic-based population. *Proc Natl Acad Sci* 93:8541–5

Ghiorzo P, Ciotti P, Mantelli M, Heouaine A, Queirolo P, Rainero ML *et al.* (1999) Characterization of ligurian melanoma families and risk of occurrence of other neoplasia. *Int J Cancer* 83:441–8

Harland M, Holland EA, Ghiorzo P, Mantelli M, Bianchi-Scarra G, Goldstein AM *et al.* (2000) Mutation screening of the CDKN2A promoter in melanoma families. *Genes Chrom Cancer* 28:45–57

Harland M, Taylor CF, Chambers PA, Kukalizch K, Randerson-Moor JA, Gruis NA *et al.* (2005) A mutation hotspot at the p14ARF splice site. *Oncogene* 24:4604–8

Hewitt C, Lee Wu C, Evans G, Howell A, Elles RG, Jordan R *et al.* (2002) Germline mutation of ARF in a melanoma kindred. *Hum Mol Genet* 11:1273–9

Holland EA, Beaton SC, Becker TM, Grulet OM, Peters BA, Rizos H *et al.* (1995) Analysis of the p16 gene, CDKN2A, in 17 Australian melanoma kindreds. *Oncogene* 11:2289–94

Hussussian CJ, Struewing JP, Goldstein AM, Higgins PA, Ally DS, Sheahan MD *et al.* (1994) Germline p16 mutations in familial melanoma. *Nat Genet* 8:15–21

Kamb A, Shattuck-Eidens D, Eeles R, Liu Q, Gruis NA, Ding W *et al.* (1994) Analysis of the p16 gene (CDKN2) as a candidate for the chromosome 9p melanoma susceptibility locus. *Nat Genet* 8:23–6

Kumar R, Smeds J, Berggren P, Straume O, Rozell BL, Akslen LA, Hemminki K (2001) A single nucleotide polymorphism in the 3′ untranslated region of the CDKN2A gene is a common in sporadic primary melanomas but mutations in the CDKN2B, CDKN2C, CDK4 and p53 genes are rare. *Int J Cancer* 95:388–93

Lilischkis R, Sarcevic B, Kennedy C, Warters A, Sutherland RL (1996) Cancer-associated missense and deletion mutations impair p16INK4 CDK inhibitory activity. *Int J Cancer* 66:249–54

Ranade K, Hussussian CJ, Sikorski RS, Varmus HE, Goldstein AM, Tucker MA *et al.* (1995) Mutations associated with familial melanoma impair p16 INK4 function. *Nat Genet* 10:114–6

Soufir N, Avril MF, Chompret A, Demenais F, Bombled J, Spatz A *et al.* (1998) Prevalence of p16 and CDK4 germline mutations in 48 melanoma-prone families in France. The French Familial Melanoma Study Group. *Hum Mol Genet* 7:209–16

Stahl S, Bar-Meir E, Friedman E, Regev E, Orenstein A, Winkler E (2004) Genetics in melanoma. *Isr Med Assoc J* 6:774–7

Walker GJ, Hussussian CJ, Flores JF, Glendening JM, Haluska FG, Dracopoli NC *et al.* (1995) Mutations of the CDKN2A/p16INK4 gene in Australian melanoma kindreds. *Hum Mol Genet* 4:1845–52

PI3-Kinase Subunits Are Infrequent Somatic Targets in Melanoma

Journal of Investigative Dermatology (2006) **126,** 1660–1663. doi:10.1038/sj.jid.5700311; published online 13 April 2006

TO THE EDITOR

The phosphatidylinositol 3-kinase (PI3K) pathway plays an important role in regulating cellular growth, proliferation, survival, and migration. The PI3K complex is a direct target of activated RAS (Rodriguez-Viciana *et al.*, 1994) and is negatively regulated by the lipid phosphatase phosphatidylinositol-3,4,5-trisphosphate 3-phosphatase (PTEN) (Stambolic *et al.*, 1998; Stahl *et al.*, 2003). The PI3K complex phosphorylates and activates the v-akt murine thymoma viral oncogene homologs (AKT) (Franke *et al.*, 1997), which are activated in many melanomas and associated with poor prognosis (Dhawan et al., 2002). Common routes to PI3K pathway activation in melanoma are mutation of neuroblastoma ras viral oncogene homolog (*NRAS*) (Albino *et al.*, 1989; vanElsas *et al.*, 1996) and deletion or mutation of *PTEN* (Herbst *et al.*, 1994; Guldberg *et al.*, 1997; Bastian *et al.*, 1998; Robertson *et al.*, 1998; Pollock *et al.*, 2002). *PTEN* inactivation is frequent in melanomas with mutation of *BRAF*, but not in melanomas with mutation of *NRAS* (Tsao *et al.*, 2004; Curtin *et al.*, 2005), suggesting that activation of the PI3K pathway cooperates with genetic alterations of the mitogen-activated protein kinase pathway in melanoma. In our previous study some melanomas showed increased expression levels of phospho-AKT but did not show mutations in *NRAS* or loss of *PTEN* (Curtin *et al.*, 2005), suggesting that additional genetic aberrations may be present upstream of AKT in the PI3K pathway. Recent reports indicate that phosphatidylinositol 3-kinase, catalytic, alpha (*PIK3CA*) is frequently mutated in other cancers (Samuels *et al.*, 2004). We sought to assess the mutational status of components of the PI3K complex in a panel of 118 primary melanomas and in 34 melanoma cell lines.

We analyzed DNA extracted from archival paraffin-embedded primary melanomas with an invasive component in which tumor cells predominated over stroma cells that in part were previously analyzed and reported (Curtin *et al.*, 2005). The study was approved by the Institutional Review Board of the University of California, San Francisco. The study compared DNA from tumors selected to obtain five groups of comparable sizes: 34 acral melanomas from the palms, soles, and subungual sites; 17 mucosal melanomas; 13 desmoplastic melanomas; 26 melanomas from chronically sun-damaged skin and 28 melanoma from skin without chronic sun damage (non-chronically sun-damaged skin). Chronically sun-damaged skin was defined by the microscopic presence or absence of marked solar elastosis of the dermis surrounding the melanomas. In all but a few cases, chronically sun-damaged skin melanoma occurred on the face and non-chronically sun-damaged skin melanoma occurred on the trunk and extremities.

Exons of interest were amplified by PCR and sequenced as described previously (Curtin *et al.*, 2005) using

Abbreviations: AKT, v-akt murine thymoma viral oncogene homolog; PI3K, phosphatidylinositol 3-kinase; PIK3CA, phosphatidylinositol 3-kinase, catalytic, alpha; PIK3CB, phosphatidylinositol 3-kinase, catalytic, beta; PIK3CD, phosphatidylinositol 3-kinase, catalytic, delta; PIK3R1, phosphatidylinositol 3-kinase, regulatory, 1; PIK3R2, phosphatidylinositol 3-kinase, regulatory, 2; PIK3R3, phosphatidylinositol 3-kinase, regulatory, 3; PTEN, phosphatidylinositol-3,4,5-trisphosphate 3-phosphatase; NRAS, neuroblastoma ras viral oncogene homolog