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Factors predicting the occurrence of germline mutations in candidate genes among patients with cutaneous malignant melanoma from South Italy

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ABSTRACT

Clinical predictors for germline mutations of candidate genes in large clinic based population of patients with cutaneous malignant melanoma (CMM) are widely awaited. Using denaturing high-performance liquid chromatography (DHPLC) analysis and DNA sequencing, 557 consecutively-collected CMM patients originating from South Italy were screened for *CDKN2A* germline mutations; subsets of them were screened for mutations in the *BRAF* and *BRCA2* genes. Seven *CDKN2A* mutations were detected in 14 (2.5%) CMM patients. Relative risk of carrying a *CDKN2A* mutation for CMM patients was demonstrated to significantly increase with the presence of familial recurrence of melanoma (risk ratio (RR) = 6.31; $p = 0.0009$), multiple primary melanomas (RR = 3.43; $p = 0.0014$), and early onset age (RR = 4.56; $p = 0.0026$). All *CDKN2A* mutations were observed in non-Sardinian patients (14/441; 3.2%), whereas *BRAF* and *BRCA2* genes were found mutated in Sardinian patients (3/116; 2.6%). Such indicators of the presence of *CDKN2A* mutations will be useful in counselling patients about undergoing genetic testing. Our findings strongly suggest that mutation rates of candidate cancer genes may deeply vary among CMM patients from different geographical areas.

1. Introduction

The incidence of cutaneous malignant melanoma (CMM) has been growing fast during the past decades; at present it is the cancer with the highest rate of increase in Caucasian populations.[1]

A number of risk factors for the development of the disease have been identified. Several evidences indicate that

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skin colour (phototype) is one of the most important factors which modifies the risk of developing melanoma. Dark-skinned populations show a substantially lower incidence of CMM than fair-skinned populations exposed to similar levels of incident sunlight.[2,3] An additional phenotypic feature which has been associated with an increased risk of CMM is the presence of multiple nevi (atypical or not).[4] Family history represents the second major risk factor. Taking into consideration different reports, 8–12% of CMM occurs in a familial setting (with a history of at least two affected family members) suggesting that genetic predisposition may indeed play an important role into the pathogenesis of melanoma.[5,6]

Silencing of tumour suppressor genes as well as activation of oncogenes have been implicated into the development of primary MM.[6,7] Germline mutations in the *CDKN2A* gene are the most common cause of inherited susceptibility to melanoma (they have been described in 10% to 50% of families from several countries).[7] The *CDKN2A* gene is located on chromosome 9p21 and encodes two distinct proteins: p16INK4A and p14ARF. Both proteins can function as tumour suppressors.[5] Studies in mouse models and humans indicate that (epi)genetic inactivation of the *CDKN2A* gene is associated with melanomagenesis.[8–10] Our group has previously reported that somatic mutations of the *BRAF* oncogene are observed in about two thirds of malignant melanomas.[11,12] The *BRAF* gene encodes a serine/threonine kinase that acts in the mitogen-activated protein kinase (MAPK) pathway, whose activation has a central role in melanocyte proliferation.[11] Recently, oncogenic *BRAF* signalling has been demonstrated to interfere with the *CDKN2A* activity. A sustained expression of the mutated BRAF protein induces p16INK4A expression and cell cycle arrest, indicating that both *BRAF* and *CDKN2A* pathways are functionally associated.[13] Finally, inherited mutations of the *BRCA2* gene give rise to a multi-site cancer phenotype which includes ocular and cutaneous melanomas in addition to the main predisposition to breast (in females and males) and ovarian cancers.[14,15]

A study from The Melanoma Genetics Consortium has indicated that the *CDKN2A* mutation penetrance varies with melanoma population incidence rates.[16] Thus, the same factors that affect population incidence of melanoma may also mediate *CDKN2A* penetrance. In Italy, different incidence rates of melanoma have been reported between the northern and southern parts of the country (standardised rates per year per 100,000 inhabitants: 9.5–11.7 in North Italy versus 4.0–4.5 in South Italy for males and 11.0–13.3 in North Italy versus 3.5–4.3 in South Italy for females[1,17]). In studies on Italian melanoma-prone families (originating from the northern and central part of the country), a high prevalence of disease-predisposing mutations has been assessed by gene susceptibility analysis.[18,19] Although most of such families were small and with few cases, about one third of them presented *CDKN2A* germline mutations.[18,19] Moreover, *CDKN2A* germline mutations have also been described in patients with more than one primary CMM without familial history.[20] These patients have an increased risk of carrying mutations in *CDKN2A*, which are detected in 8% to 15% of patients presenting multiple primary melanoma and unselected for family history.[20–22]

In recent years, numerous studies have assessed the *CDKN2A* mutation prevalence in various subsets of sporadic and familial CMM patients; few of them have evaluated the predictors for the occurrence of *CDKN2A* mutations in a large clinic based population. Factors predictive for the presence of *CDKN2A* germline mutations in patients with melanoma are widely awaited in order to provide more accurate guidance to patients and their families about undergoing genetic testing.

Our aim in the present work was to assess the likelihood of identifying *CDKN2A* mutations in cases who present to high-risk cancer evaluation clinics by studying a large series of consecutively-collected clinical CMM patients. Moreover, molecular analysis was also performed in order to identify any correlation between genetic alterations and phenotypic parameters (age at diagnosis, tumour primary site, histopathological features, disease stage, cancer family history) in these patients. Finally, the study evaluated the prevalence of two additional melanoma candidate genes, *BRAF* and *BRCA2*, providing the first opportunity to make comparisons with data from different Italian geographical areas.

2. Materials and methods

2.1. *Cases and controls*

Five hundred and fifty-seven patients with histologically-proven diagnosis of CMM were included into the study. To avoid any bias, melanoma patients were consecutively collected from January 2001 to September 2005; they were included regardless of age at diagnosis, family history status, and disease features. In our previous work,[12] we carried out mutation analysis of the *CDKN2A* and *BRAF* genes in a large collection of Italian melanoma patients. In particular, about half of the present cohort (268 patients) has been already tested for *CDKN2A* and *BRAF* mutations in our previous study.

Clinical and pathological features such as histological classification (including Breslow thickness and Clark level of invasion) and disease stage at diagnosis were confirmed by medical records, review of pathologic material, and/or pathology reports.

Familial recurrence of melanoma was ascertained through specific cancer evaluation programs at both the National Tumour Institute of Naples (which represents the principal institution accounting for cancer patients from South Italy) and the University of Sassari (accounting for the majority of cancer patients from Sardinia). Family data, including demographic characteristics (sex, date and place of birth), occurrence of melanoma and any other cancer, were collected by using the same only questionnaire to interview probands about their first-, second-, and third-degree relatives. Melanoma families were identified according to standardised criteria[6,7]: *a*) at least three affected members, or *b*) two affected members and presence of at least one of the following subcriteria: i) at least one affected member younger than 50 years at onset, or ii) a case of pancreatic cancer in a first- or second-degree relative, or iii) one affected member with multiple primary melanomas. After patients were informed about aims and limits of the study, blood samples were obtained with their written consent.

The study was reviewed and approved by the ethical review boards of both the National Tumour Institute of Naples and the University of Sassari.

2.2. Mutation screening of candidate genes

For mutation analysis, genomic DNA was isolated from peripheral blood samples, using standard methods. The full coding sequence and splice junctions of *CDKN2A* gene were screened for mutations by direct sequencing, using an automated fluorescence-based cycle sequencer (ABIPRISM 3100, Applied Biosystems, Foster City, CA). Primer sets and protocols for polymerase chain reaction (PCR) assays were as previously described.[12] Subsets of patients originating from different geographical areas within South Italy were screened for mutations in the *BRAF* and *BRCA2* genes, as previously reported.[12,23] Briefly, PCR products corresponding to all exons and intron-exon boundaries of such two genes were analysed by denaturing high-performance liquid chromatography (DHPLC). All PCR products with abnormal DHPLC profiles were sequenced as above. To avoid any bias, mutation analysis for *BRCA2* gene was performed on first 100 consecutively-collected CMM samples (11 of them were excluded from the series because of DNA degradation). Screening for *BRAF* gene was incomplete in a fraction of patients (120/557; 22%) due to the low amount of available genomic DNA.

To evaluate the prevalence of each gene variant in a control population, 100 unrelated healthy individuals (corresponding to 200 control chromosomes), originating from the same geographical areas and with no family history for cancer, were used as controls and screened for each sequence variation identified.

2.3. Statistical analysis

Statistical correlation between *CDKN2A* mutations and clinical or pathological variables was performed by chi-square test. Characteristics included the following: sex, age at diagnosis, anatomical site of primary melanoma, stage of disease, presence of multiple CMM, presence of familial recurrence of the disease. Odds ratios of carrying *CDKN2A* mutations were estimated by the logistic regression model and are reported with 95% confidence interval (95% CI). Features for the relative risk calculation were analysed as dichotomous variables (presence *versus* absence). The exact coefficient for sample proportion analysis was performed to determine all significant parameters (below 0.05 level). All analyses were performed using the statistical package SPSS/7.5 per Windows.

3. Results

Genomic DNA from 557 consecutively-collected CMM patients was screened for germline mutations of the *CDKN2A* gene. PCR products corresponding to the coding exons and intron-exon junctions were analysed by direct sequencing using an automated approach. In our series, 29/557 (5.2%) CMM patients had a familial recurrence of malignant melanoma (presence of at least two affected members in the family). Majority (332/557; 59.6%) of patients included into the study were females; median age was 46 years, with a range from 16 to 85 years. About one fifth (116/557; 20.8%) of patients originated from Sardinia; however, no substantial difference was observed in patients' characteristics between the Sardinian and the South Italian series.

Seven germline mutations were found in the coding sequence of *CDKN2A* among 14/557 (2.5%) CMM patients (Table 1). Majority of mutations (9/14; 64%) were located in exon 1. The variant A109V has not been previously reported in the Human Gene Mutation Database at http://archive.uwcm.ac.uk. All mutations were absent in normal genomic DNA from 100 unrelated healthy individuals (corresponding to 200 control chromosomes).

As shown in Table 2, the proportion of patients carrying *CDKN2A* germline mutations varied when we analysed different phenotypic parameters. According to the selection criteria (see Methods), *CDKN2A* mutations were more frequent in patients with familial history of melanoma (5/29; 17.2%) compared to patients without (9/528; 1.7%) (Table 2). Moreover, *CDKN2A* mutations were more frequent in patients with two or more synchronous or asynchronous melanomas (5/34; 14.7%) than in patients with a single melanoma (9/523; 1.7%) (Table 2). Finally, age at diagnosis $\leqslant$45 years was significantly correlated with the presence of a *CDKN2A* mutation ($P = 0.0026$; Table 2). Nevertheless, the mean age of onset was significantly lower in carriers of mutations (33.7 years) compared to non-carriers (46.9 years; $P = 0.003$). In a subset of 316 patients whose information about the total number of skin nevi were available, presence of multiple nevi did not predict the occurrence of CDKN2A mutations [2/53 (3.8%) versus 10/263 (3.8%) CDKN2A-positive cases among patients with and without multiple nevi, respectively; data not shown]. No other parameter in CMM patients (sex, primary tumour location, Breslow thickness, Clark level of invasion, stage of disease) was statistically correlated with the presence of *CDKN2A* mutations (Table 2).

Table 1 – CDKN2A germline mutations

Exon	Nucleotide	Codon	Base change	Amino acid change	Designation	Positive cases (%)
1	35	12	C to A	Ser to Stop	S12X	1 (0.18)
1	71	24	G to C	Arg to Pro	A24P	3 (0.54)
1	76	26	G to T	Glu to Stop	E26X	1 (0.18)
1	106	36	G to A	Ala to Thr	A36T	4 (0.72)
2	176	59	T to G	Val to Gly	V59G	3 (0.54)
2	301	101	G to T	Gly to Trp	G101W	1 (0.18)
2	326	109	C to T	Ala to Val	A109V	1 (0.18)

Percentage is reported for the entire series of 557 analysed patients.

Table 2 – Frequency of CDKN2A germline mutations according to patients' characteristics

Subgroups (No. of patients)	Cases positive to CDKN2A mutations		P
	No.	%	
All patients (557)	14	2.5	
Sex			
Male (225)	5	2.2	0.2382
Female (332)	9	2.7	
Site of primary MM			
Head & neck (45)	1	2.2	0.4363
Trunk (243)	6	2.5	
Limbs (269)	7	2.6	
Number of primary MM			
Single (523)	9	1.7	0.0014
Multiple (34)	5	14.7	
Breslow thickness			
<1 mm (271)[a]	7	2.6	0.3111
1–2 mm (139)	3	2.2	
>2 mm (147)	4	2.7	
AJCC Stage			
0–I (282)	7	2.5	0.5792
II (190)	5	2.6	
III–IV (85)	2	2.4	
Age at diagnosis			
⩽35 years (142)	8	5.6	0.0067
36–45 years (121)	3	2.5	
46–55 years (115)	1	0.9	
⩾56 years (179)	2	1.1	
⩽45 years (263)	11	4.2	0.0026
⩾46 years (294)	3	1.0	
Familial history of MM			
No familial recurrence (528)	9	1.7	0.0009
Familial recurrence (29)	5	17.2	

Disease stage was defined according to the recent American Joint Committee on Cancer (AJCC) guidelines.
P: chi-squared test; two tailed; 95% confidence interval.
a Including 23 cases with *in situ* CMM.

Logistic regression multivariate analysis was performed on the totality of patients in order to estimate the role of the different disease parameters (sex, diagnosis age, primary site, Breslow, Clark, AJCC stage, family history of melanoma) on likelihood of carrying CDKN2A germline mutations. The familial recurrence of melanoma (risk ratio (RR), 6.31; 95% CI, 2.96 to 10.83), the presence of multiple primary melanomas (RR, 3.43; 95% CI, 1.84 to 5.42), and the early age of onset (RR, 4.56; 95% CI, 2.23 to 6.74) remained statistically independent factors predicting the occurrence of CDKN2A germline mutations in CMM patients (Table 3).

Considering the patients' geographical origin, all *CDKN2A* mutations were observed in CMM cases from the other southern Italian regions outside of the Sardinia island [14/441 (3.2%) non-Sardinian patients versus 0/116 Sardinian patients] (Table 4). Given the potential differences between the Sardinian series and the other South Italian samples, evaluations conducted excluding the Sardinian patients confirmed the factors (familial recurrence of melanoma, multiple primary melanoma, early diagnosis) predicting the occurrence of a *CDKN2A* mutation (data not shown).

To evaluate whether additional candidate genes might be involved in the melanoma susceptibility, prevalence of germline mutations in *BRAF* and *BRCA2* genes (the other two major genes related to melanoma pathogenesis) was assessed in subsets of patients originating from different geographical areas within South Italy (see Methods).

Mutation screening for all coding regions and splice boundaries of *BRAF* and *BRCA2* genes was performed by DHPLC analysis on germline DNAs; all PCR products presenting an abnormal denaturing profile in comparison to the normal controls were sequenced using an automated approach. Three (2.6%) out of 116 Sardinian patients were found to carry germline mutations in the coding region of either *BRAF* or *BRCA2* genes (Table 4). Again, all three mutations were absent in normal genomic DNA from 100 unrelated healthy individuals (corresponding to 200 control chromosomes). In particular, two of these mutations were found in *BRCA2* (1.7%; BRCA2-8765delAG, and BRCA2-Ser2835Pro) and the remaining one in *BRAF* (0.9%; Met116Arg) (Table 4). The mutation BRCA2-8765delAG has been previously described by our group as a frameshift mutation with founder effect in North Sardinia.[24] No germline mutation in either *BRAF* or *BRCA2* genes was observed in 321 and 89 non-Sardinian patients, respectively (Table 4). Considering the cancer family history, patients with the BRCA2 or BRAF mutations presented recurrence of non-melanoma cancers in their families (BRCA2-8765delAG: two family members with breast carcinoma; BRCA2-Ser2835Pro: two with gastro-intestinal cancer and one with prostate carcinoma; BRAF-Met116Arg: two with breast carcinoma and one with colorectal cancer).

Table 3 – Multivariate analysis of different parameters for likelihood of carrying CDKN2A germline mutations

Characteristic	Risk ratio	95% CI	P
Sex (male versus female)	0.71	0.43–1.18	0.174
Multiple melanoma (presence versus absence)	3.43	1.84–5.42	<0.01
Breslow thickness (<2 mm versus ⩾2 mm)	0.98	0.46–1.91	0.673
Clark level (I–II versus III–V)	1.01	0.98–1.04	0.180
Stage of disease (localised [0–II] versus metastatic [III–IV])	0.93	0.78–1.07	0.331
Age at diagnosis (<45 versus >45 years)	4.56	2.23–6.74	<0.01
Familial recurrence (presence versus absence)	6.31	2.96–10.83	<0.001

CI, confidence interval.

Table 4 – Germline mutations in candidate genes according to patients' origin

Analysed gene	No. of analysed patients	Cases positive to germline mutations	
		No.	%
Non-Sardinian patients			
CDKN2A	441	14	3.2
BRAF	321	0	0
BRCA2	89	0	0
Sardinian patients			
CDKN2A	116	0	0
BRAF	116	1[a]	0.9
BRCA2	116	2[b,c]	1.7

Age at diagnosis:
a 68 years.
b 40 years.
c 37 years.

Fig. 1 – Distribution of familial cases with germline mutations in candidate cancer genes. The geographical area with the white colour corresponds to South Italy. Dots indicate the origin of patients carrying germline mutations (in black, *CDKN2A* mutations; in grey, *BRAF*/*BRCA2* mutations).

In Fig. 1, the geographical distribution of all cases carrying germline mutations in the three candidate genes (*CDKN2A*, *BRAF*, and *BRCA2*) analysed in the present study is reported.

4. Discussion

A mutation analysis of *CDKN2A*, which is the main gene associated with melanoma susceptibility, was performed on germline DNA from 557 melanoma patients originating from different regions of South Italy. Subsets of patients were also screened for germline mutations in *BRAF* gene, which we previously demonstrated to be quite exclusively mutated at somatic level in two thirds of malignant melanomas and at lower frequency in a wide range of human cancers[11,12], as well as in the *BRCA2* gene, which seems to play a major role in predisposition to different types of cancer including melanoma.[14,15,25] In the present study, prevalence of germline mutations in such candidate genes has been extensively evaluated in CMM patients with the same geographical origin. In this series, disease information and presence of familial recurrence of melanoma for each patient was ascertained through well-defined clinical criteria.

Nearly 2.5% of the patients studied were carriers of germline mutations in the *CDKN2A* gene. Also taking into consideration some lack of sensitivity of our screening assay to reduce the number of positive cases, a very low frequency of germline mutations in such a main candidate gene was observed among southern Italian melanoma patients. The likelihood of finding a mutation in *CDKN2A* significantly increased with the presence of familial history of melanoma (RR, 6.31), the diagnosis of multiple melanoma (RR, 3.43), and the early disease onset (age at diagnosis <45 years; RR, 4.56) (see Table 3). In details, we found that *CDKN2A* mutations were more frequent in patients with at least two affected family members (17.2%) compared with those without familial CMM recurrence (1.7%), in patients with two or more melanomas (14.7%) compared with those with only one melanoma (1.7%), and in patients with diagnosis age $\leqslant$45 years (4.2%) compared with those with diagnosis age $\geqslant$45 years (1.0%; see Table 2). In other words, patients with such features presented a frequency of *CDKN2A* mutations 4-fold to about 10-fold higher than that of patients without.

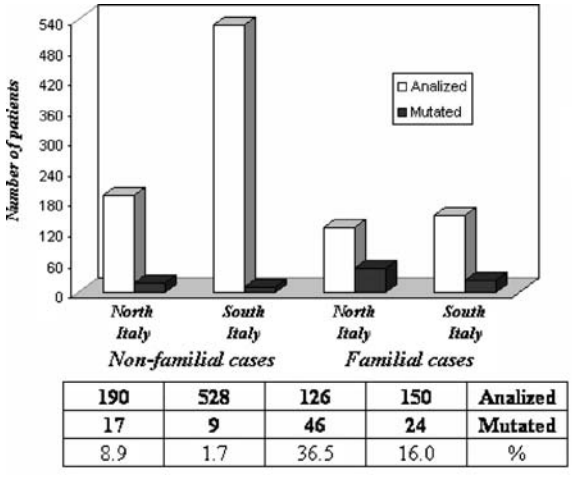
Our study also suggests the existence of some phenotypic difference between CMM patients who are carriers of *CDKN2A* mutations and those who are not. For instance, the age of onset was significantly lower in patients with mutations (33.7 years) compared with non-carriers (46.9 years; $P = 0.003$). Conversely, the age of onset was not statistically different in patients belonging to melanoma families and patients without familial melanoma history, regardless of mutation status. This is consistent with studies in other populations.[22,26–28] In particular, a clinic-based population study have indicated that such clinical features (early age of onset, multiple primary tumours, and multiple affected family members) are tightly associated with predisposing genetic mutations in familial CMM cases.[29] Nevertheless, a study based on a logistic regression model (MELPREDICT) among familial melanoma cases in order to estimate probability of the proband being a mutation carrier has further demonstrated that proband age at diagnosis, number of proband primaries, and number of additional family primaries are most closely associated with germline mutations.[30] Our findings confirmed the predictive value of such indicators more in general, among unselected melanoma patients (for onset age and multiple primary tumours, regardless of the cancer family history of each patient).

Melanocytic transformation is thought to occur by sequential accumulation of genetic alterations. Several studies examining the status of *CDKN2A* have indicated that this gene is altered in the large majority of melanoma cell lines,

through loss of p16INK4A function, but in much lower fractions of uncultured melanomas (either primary tumours or metastases).[31–33] Moreover, evaluation of the prevalence of such mutations within different geographical areas indicates that *CDKN2A* penetrance varies according to the variation of the population incidence rates for melanoma.[16]

As schematically represented in Fig. 2, we made comparisons between prevalences of *CDKN2A* germline mutations within different Italian regions, pooling data from previous publications[18,19,34–36] and from the present study. In contrast to a higher frequency (about 9%) of *CDKN2A* germline mutations observed in non-familial cases from North Italy, our findings (prevalence of 1.7%) among the same type of patients from South Italy strongly suggest that discrepancy in *CDKN2A* mutation frequency may be due to patients' origin and/or to the different 'genetic background' of populations. Nevertheless, data on familial melanomas (36.5% versus 16.0% *CDKN2A* germline mutation carriers between North and South Italy, respectively; see Fig. 2) are also consistent with the hypothesis that the *CDKN2A* mutation penetrance varies with melanoma population incidence rates. In fact, either the standardised incidence rates of melanoma (roughly, 12 per 100,000 inhabitants in North Italy versus 4 per 100,000 inhabitants in South Italy[1,17]) or the prevalence of familial CMM cases (9.8% in patients from North Italy[18,19] versus 5.2% in those from South Italy of the present study) are significantly different in these two geographical areas. Thus, the same factors that affect population incidence of melanoma may also mediate *CDKN2A* penetrance.

Overall, these data seem to indicate that genetic factors predisposing to CMM may be more heterogeneous in South than in North Italy. Patients' origin may account for different mutation rates in candidate cancer genes. Indeed, all *CDKN2A* germline mutations here identified were observed in non-Sardinian patients only (14/441; 3.2%), with no *CDKN2A* mutation carrier into the series from Sardinia. Conversely, germline mutations in *BRAF* and *BRCA2* genes were found in Sardinian patients only (altogether, 3/116; 2.6%), with no additional alteration in the remaining cases from South Italy (see Table 4).



190	528	126	150	Analized
17	9	46	24	Mutated
8.9	1.7	36.5	16.0	%

Fig. 2 – Prevalence of *CDKN2A* germline mutations in North and South Italy. Familial cases were classified according to standardised selection criteria (see Methods).

Our findings strongly suggest that mutation frequency for any candidate cancer gene needs to be evaluated in each geographical area.

Knowledge of the likelihood of detecting *CDKN2A* mutations has particular relevance to address patients and their families to clinical screening as well as to provide clinical recommendations for *CDKN2A* genetic tests, affecting patient decisions and cost/effectiveness estimates. The present study represents one of the efforts toward the assessment of the role and the clinical implication of *CDKN2A* mutations in unselected patients with malignant melanoma. This information should therefore be considered when counselling patients and their families about undergoing genetic testing and, in particular, when considering management strategies for individuals with documented mutations. In addition, analysis of these genotype/phenotype relationships will result in better knowledge of penetrance and clinical variability of the disease.

More in general, the molecular prediction of melanoma risk through identification of genetic mutation carriers and the existence of improved clinical screening by epiluminiscence microscopy and digital follow-up may indeed result in the detection of early melanomas. Mutational screening and regular and thorough dermatologic screening of relatives of individuals harbouring germline mutations in candidate genes could even improve prevention and surveillance strategies of melanoma through early identification of the pigmented skin lesions at risk of malignant transformation.

Conflict of interest statement

None declared.

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REFERENCES

1. Parkin DM, Whelan SL, Ferlay J, et al. *Cancer incidence in five continents*, Vol. VIII. Lyon, France: IARC Press; 2003.
2. Gilchrest BA, Eller MS, Geller AC, Yaar M. The pathogenesis of melanoma induced by ultraviolet radiation. *New Engl J Med* 1999;**340**:1341–8.
3. Jhappan C, Noonan FP, Merlino G. Ultraviolet radiation and cutaneous malignant melanoma. *Oncogene* 2003;**22**:3099–112.
4. Thompson JF, Scolyer RA, Kefford RF. Cutaneous melanoma. *The Lancet* 2005;**365**:687–701.
5. Haluska FG, Hodi FS. Molecular genetics of familial cutaneous melanoma. *J Clin Oncol* 1998;**16**:670–82.

6. Bataille V. Genetic epidemiology of melanoma. *Eur J Cancer* 2003;**39**:1341–7.
7. Hayward NK. Genetics of melanoma predisposition. *Oncogene* 2003;**22**:3053–62.
8. Wang YL, Uhara H, Yamazaki Y, et al. Immunohistochemical detection of CDK4 and p16INK4 proteins in cutaneous malignant melanoma. *Br J Dermatol* 1996;**134**:269–75.
9. Sharpless E, Chin L. The INK4a/ARF locus and melanoma. *Oncogene* 2003;**22**:3092–8.
10. Bennett DC. Human melanocyte senescence and melanoma susceptibility genes. *Oncogene* 2003;**22**:3063–9.
11. Davies H, Bignell GR, Cox C, et al. Mutations of the BRAF gene in human cancer. *Nature* 2002;**417**:949–54.
12. Casula M, Colombino M, Satta MP, et al. BRAF gene is somatically mutated but does not make a major contribution to malignant melanoma susceptibility. *J Clin Oncol* 2004;**22**:286–92.
13. Michaloglou C, Vredeveld LCW, Soengas MS, et al. $BRAF^{E600}$-associated senescence-like cell cycle arrest of human naevi. *Nature* 2005;**436**:720–4.
14. The Breast Cancer Linkage Consortium. Cancer risks in BRCA2 mutation carriers. *J Natl Cancer Inst* 1999;**91**:1310-1316.
15. Thompson D, Easton D. Breast Cancer Linkage Consortium: Variation in cancer risks, by mutation position, in *BRCA2* mutation carriers. *Am J Hum Genet* 2001;**68**:410–9.
16. Bishop DT, Demenais F, Goldstein AM, et al. Geographical variation in the penetrance of CDKN2A mutations for melanoma. *J Natl Cancer Inst* 2002;**94**:894–903.
17. Budroni M, Tanda F. Registro Tumori della Provincia di Sassari I tumori in Sardegna negli anni novanta. Tipografia Moderna, Sassari, Italy 2002;9-22.
18. Mantelli M, Barile M, Ciotti P, et al. High prevalence of the G101W germline mutation in the CDKN2A (P16(ink4a)) gene in 62 Italian malignant melanoma families. *Am J Med Genet* 2002;**107**:214–7.
19. Della Torre G, Pasini B, Frigerio S, et al. CDKN2A and CDK4 mutation analysis in Italian melanoma-prone families: functional characterization of a novel CDKN2A germ line mutation. *Br J Cancer* 2001;**85**:836–42.
20. Monzon J, Liu L, Brill H, et al. Mutations in multiple primary melanomas. *N Engl J Med* 1998;**338**:879–87.
21. Hashemi J, Platz A, Ueno T, et al. CDKN2A germline mutations in individuals with multiple cutaneous melanomas. *Cancer Res* 2000;**60**:6864–7.
22. Puig S, Malvehy J, Badenas C, et al. Role of the *CDKN2A* locus in patients with multiple primary melanomas. *J Clin Oncol* 2005;**23**:3043–51.
23. Palomba G, Pisano M, Cossu A, et al. Spectrum and prevalence of *BRCA1* and *BRCA2* germline mutations in Sardinian breast cancer patients through a hospital-based screening. *Cancer* 2005;**104**:1172–9.
24. Palmieri G, Palomba G, Cossu A, et al. BRCA1 and BRCA2 germline mutations in Sardinian breast cancer families and their implications for genetic counseling. *Ann Oncol* 2002;**13**:1899–907.
25. Liede A, Karlan BY, Narod SA. Cancer risks for male carriers of germline mutations in BRCA1 or BRCA2: a review of the literature. *J Clin Oncol* 2004;**22**:735–42.
26. MacKie RM, Andrew N, Lanyon WG, et al. CDKN2A germline mutations in UK patients with familial melanoma and multiple primary melanomas. *J Invest Dermatol* 1998;**111**:269–72.
27. Piepkorn M. Melanoma genetics: An update with focus on the CDKN2A(p16)/ARF tumor suppressors. *J Am Acad Dermatol* 2000;**42**:705–22.
28. Rutter JL, Bromley CM, Goldstein AM, et al. Heterogeneity of risk for melanoma and pancreatic and digestive malignancies: a melanoma case-control study. *Cancer* 2004;**101**:2809–16.
29. FitzGerald MG, Harkin DP, Silva-Arrieta S, et al. Prevalence of germ-line mutations in p16, p19ARF, and CDK4 in familial melanoma: analysis of a clinic-based population. *Proc Natl Acad Sci U S A* 1996;**93**:8541–5.
30. Niendorf K, Goggins W, Yang G, et al. MELPREDICT: A logistic regression model to estimate CDKN2A carrier probability. J Med Genet 2005, [Epub ahead of print].
31. Pollock PM, Pearson JV, Hayward NK. Compilation of somatic mutations of the CDKN2 gene in human cancers: non-random distribution of base substitutions. *Genes Chromosom Cancer* 1996;**15**:77–88.
32. Smith-Sorenson B, Hoving E. CDKN2A [P16(INK4A)] somatic and germline mutations. *Hum Mutat* 1996;**7**:294–303.
33. Foulkes WD, Flanders TY, Pollock PM, Hayward NK. The CDKN2A (p16) gene and human cancer. *Molec Med* 1997;**3**:5–20.
34. Casula M, Ascierto PA, Cossu A, et al. Mutation analysis of candidate genes in melanoma-prone families: evidences of different pathogenetic mechanisms at chromosome 9p21. *Melanoma Res* 2003;**13**:571–9.
35. Mantelli M, Pastorino L, Ghiorzo P, et al. Frequency of CDKN2A mutation in Ligurian non-familial melanoma patients. Third Research Meeting on Melanoma, Milan, Italy. *Melanoma Res* 2004;**14**:77–84.
36. Mantelli M, Pastorino L, Ghiorzo P, et al. Early onset may predict G101W CDKN2A founder mutation carrier status in Ligurian melanoma patients. *Melanoma Res* 2004;**14**:443–8.