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Clinical and histologic characteristics of malignant melanoma in families with a germline mutation in CDKN2A

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Abstract

Background:
About 10% of cutaneous malignant melanomas (CMM) occur in individuals with a family history of melanoma. In 20% to 40% of melanoma families germline mutations in CDKN2A are detected. Knowledge of the clinicohistologic characteristics of melanomas and patients from these families is important for optimization of management strategies, and may shed more light on the complex interplay of genetic and environmental factors in the pathogenesis of melanoma.

Objective:
We sought to investigate the clinical and histologic characteristics of CMM in CDKN2A-mutated families.

Methods:
Clinical and histologic characteristics of 182 patients with 429 CMM from families with a founder mutation in CDKN2A (p16-Leiden mutation) were compared with 7512 patients with 7842 CMM from a population-based cancer registry.

Results:
Patients with p16-Leiden had their first melanoma 15.3 years younger than control patients. The 5-year cumulative incidence of second primary CMM was 23.4% for patients with p16-Leiden compared with 2.3% for control patients. The risk of a second melanoma was twice as high for patients with p16-Leiden who had their first melanoma before age 40 years, compared with older patients with p16-Leiden. Unlike control patients, there was no body site concordance of the first and second melanoma in patients with p16-Leiden and multiple primary melanomas. Patients with p16-Leiden had significantly more superficial spreading, and less nodular and lentiginous melanomas.

Limitations:
Ascertainment of patients with p16-Leiden was family based. The study was performed in families with a founder mutation, the p16-Leiden mutation.

Conclusion:
Our findings are consistent with a pathogenic pathway of melanoma development from nevi, starting early and ongoing throughout life, and not related to chronic sun exposure.

Introduction

Between 6% and 14% of cases of primary cutaneous malignant melanoma (CMM)) have been reported to occur in individuals with a family history of melanoma.[1] In these families two major melanoma susceptibility genes have been identified so far. The oncogene CDK4 (MIM# 123829) has been found in a few melanoma families (estimated 2%).[2] Germline mutations in CDKN2A (MIM# 600160) are far more prevalent and are found in approximately 20% to 40% of melanoma families.[3] CDKN2A encodes two distinct proteins: p16INK4 and p14ARF, which both function as tumor suppressors. The penetrance of CDKN2A mutations for melanoma has been estimated to be 0.67 by the age of 80 years.[4] In The Netherlands the p16-Leiden mutation (c.225-243del19) is the most prevalent CDKN2A germline mutation.[5]

Several studies have reported that patients with melanoma and a CDKN2A mutation have an earlier age of onset and have an increased risk of multiple primary melanomas (MPM).[6-9] The purpose of this study was to further substantiate and expand the knowledge of the clinical and histologic characteristics of patients with melanoma from CDKN2A-mutated families. Knowledge of these features may not only provide useful information for clinicians, but can also shed more light on the complex interplay of genetic and environmental factors in the pathogenesis of melanoma.

We investigated the clinical and histologic characteristics of malignant melanoma in families with the p16-Leiden mutation, by comparing 182 patients with melanoma from p16-Leiden families who had 429 melanomas with patients and melanomas from a population-based cancer registry.

Methods

P16-Leiden cases were collected from proven p16-Leiden-mutated families that were registered at The Netherlands Foundation for the Detection of Hereditary Tumors (NFDHT). The organization and methods of the NFDHT have been published elsewhere.[10,11] In brief, physicians from all parts of The Netherlands refer families suspected for familial melanoma to the registry. Genealogic studies are performed and all reported malignancies are verified by medical records. If familial melanoma is confirmed clinically, the registry monitors the continuity of the surveillance program for relatives and collects follow-up data on the results of surveillance and pathologic examination. In 2007 the NFDHT database contained 51 p16-Leiden families with 194 patients with confirmed primary CMM since 1970. The majority of patients (n = 144, 74%) had been treated for at least one of their melanomas at the Department of Dermatology at the Leiden University Medical

Center, which is a tertiary referral center for familial melanoma. Most data on patient mutation status for the study were collected at this department as described elsewhere.[12] In addition we used results from clinical genetic testing, which the NFDHT receives if patients consent.

Twelve melanoma patients that had tested negative for the p16-Leiden mutation in their family were excluded from the study. The remaining 182 patients consisted of 127 proven mutation carriers and 55 untested patients (from proven p16-Leiden families). As 8.6% (12/139) of gene-tested patients tested negative for the p16-Leiden mutation, we expected about 5 phenocopies among the 55 untested patients. We found this acceptable and decided to include the untested patients as p16-Leiden patients in the study. For each patient, follow-up data were collected on subsequent melanomas and life status.

Control patients were obtained from the Leiden Cancer Registry (LCR). The LCR is a population-based registry of all newly diagnosed malignancies, which covers the western part of The Netherlands with a population of approximately 1.7 million inhabitants. The registry has (near) complete coverage since 1989. For new patients in the registry, malignancies diagnosed before the start of the registry are also recorded. All patients with histologically confirmed primary cutaneous melanoma up to September 2007 were selected from the cancer registry. The patients known to be members of p16-Leiden families were excluded from the LCR database. Survival data of the control patients were obtained from the Central Bureau for Genealogy and from the municipal registries, which keep records of all deceased persons in The Netherlands. Survival data were completed until January 2006. The LCR contained a total of 7512 eligible patients. For control patients no data on CDKN2A mutation status were available. For all included patients, data were gathered concerning date of birth and gender. For all melanomas, data were collected concerning date of diagnosis, histologic type, and body site.

Statistical analyses

Body site was subdivided into head and neck, trunk, upper extremities, lower extremities, and not recorded. Histologic type was categorized as superficial spreading melanoma (SSM) or melanoma in situ (Mis), nodular melanoma (NM), lentigo maligna (LM) melanoma (LMM) or LM, and acral lentiginous melanoma (ALM) or ALM in situ. Other histologic types and melanomas that were not otherwise specified were excluded from all analyses that included the variable histologic type. In some analyses a dichotomous covariate was included to distinguish invasive from in situ melanomas. Age and year of diagnosis were analyzed as continuous variables. In some analyses age was divided in two categories. To distinguish the two study populations we used the term "p16-Leiden status", coded 1 for patients with p16-Leiden, and 0 for control patients. This term refers to being a member of a p16-Leiden-mutated family, rather than personal mutation status (see above).

Differences in gender distribution and age at diagnosis were calculated with the Pearson x^2 and Student t test, respectively. The cumulative incidence of second primary melanomas was calculated using a competing risk analysis[13] accounting for death as competing risk. Survival times were calculated from the date of first melanoma to the date of second melanoma, death, or last follow-up. For control patients, January 2006 was considered as the end of follow-up as data on life status were only available until this date. Melanomas diagnosed after January 2006 were therefore excluded from the competing risk analyses.

A Cox proportional hazard model was used to compare the hazard of developing a second primary melanoma in the two study populations, and to study risk factors for developing a second melanoma in the two populations separately. Because the risk of developing a second melanoma was age dependent in both populations, separate hazard ratios (HRs) were calculated for age below and above 40 years. Equality of HRs across age groups was tested using Cox regression with p16-Leiden status, age group, and their interaction. To identify risk factors for a second primary melanoma, the following covariates were tested in univariate analyses: age of diagnosis (<40 vs >40 years), age of diagnosis (<60 vs >60 years), gender, year of diagnosis, tumor type (SSM/Mis, NM, LMM/LM, ALM/ALM in situ), tumor localization (lower extremities, head/neck, trunk, upper extremities), and invasiveness of the first melanoma (in situ vs invasive melanoma). Covariates with a P value less than .10 in the univariate analyses were included in the multivariate analyses.

Body site concordance of the first and second primary melanoma was calculated with Cohen k statistics. To compare concordance in patients with p16-Leiden and control patients an analysis of variance was performed.
For comparison of tumor characteristics of p16-Leiden cases and control patients, a multivariate logistic regression analysis was performed with robust SEs to correct for within-patient correlations in relatives with MPM. Comparison of the age at diagnosis according to body site pattern in the two study populations was done by means of a 3-way analysis of variance.

All analyses were performed with software (SPSS 14.0, SPSS Inc, Chicago, IL, and R 2.5.1, R Development Core Team, Vienna, Austria). Statistical significance was determined at a = .05, and all tests for statistical significance were two-sided.

Results

Patient characteristics

In total 182 patients with p16-Leiden melanoma and 429 CMM, and 7512 control patients with melanoma and 7842 CMM were included in the analyses. Patient characteristics are presented in Table 1. The gender distribution was similar in both groups. Age at diagnosis

Table 1 Patient and tumor characteristics

	p16-Leiden melanoma patients (n = 182)		Control melanoma patients (n = 7512)		p-value
p16-Leiden mutation status					
Positive	127	(69.8%)	N/A		
Not tested	55	(30.2%)	N/A		
Gender					
Male	80	(44.0%)	2977	(39.6%)	0.24
Female	102	(56.0%)	4535	(60.4%)	
Age					
Mean (SD), Male	40.5 yrs	(13.0)	55.3 yrs	(16.7)	< 0.001
Mean (SD), Female	37.9 yrs	(13.5)	53.7 yrs	(18.0)	< 0.001
Mean (SD), All	39.0 yrs	(13.3)	54.3 yrs	(17.5)	< 0.001
Total No. of melanomas	429		7842		
No. of melanomas / patient					
1	108	(59.3%)	7239	(96.4%)	< 0.001
2	30	(16.5%)	236	(3.1 %)	
3-5	27	(14.8%)	34	(0.5%)	
> 5	17	(9.3%)	3	(0%)	
Cum. Incidence of second melanoma					
1-Year incidence	8.5%		1.0%		
2-Year incidence	12.2%		1.5%		
5-Year incidence	23.4%		2.3%		
10-Year incidence	34.8%		3.1%		
20-year incidence	41.4%		4.5%		
Tumor type					< 0.001
SSM/Mis	338	(88.9%)	4205	(66.2%)	
NM	29	(7.6%)	1129	(17.8%)	
LMM/LM	8	(2.1%)	973	(15.3%)	
ALM/ALMis	5	(1.3%)	47	(0.7%)	
Other	1		275		
NOS	48		1213		
Location					< 0.001
Male					
Head & Neck	27	(14.4%)	720	(23.4%)	
Trunk	102	(54.3%)	1396	(45.4%)	
Upper extremities	26	(13.8%)	486	(15.8%)	
Lower extremities	33	(17.6%)	473	(15.4%)	
Unknown	1		38		

Table 1 Continued

	p16-Leiden melanoma patients (n = 182)		Control melanoma patients (n = 7512)		p-value
Female					
Head & Neck	26	(10.9%)	828	(17.8%)	
Trunk	70	(29.4%)	1186	(25.4%)	
Upper extremities	45	(18.9%)	963	(20.7%)	
Lower extremities	97	(40.8%)	1684	(36.1%)	
Unknown	2		68		
Invasiveness					
In situ	94	(22.0%)	1641	(20.9%)	0.61
Invasive	334	(78.0%)	6201	(79.1%)	
Unknown	1				

N/A, not applicable; SD, standard deviation; MPM-patients, multiple primary melanoma patient; Cum. incidence, cumulative incidence; SSM/Mis, Superficial Spreading Melanoma or Melanoma in situ; NM, Nodular Melanoma; LMM/LM, Lentigo Maligna Melanoma or Lentigo Maligna; ALM/ALMis, Acrolentiginous Melanoma or Acrolentiginous Melanoma in situ; NOS, not otherwise specified

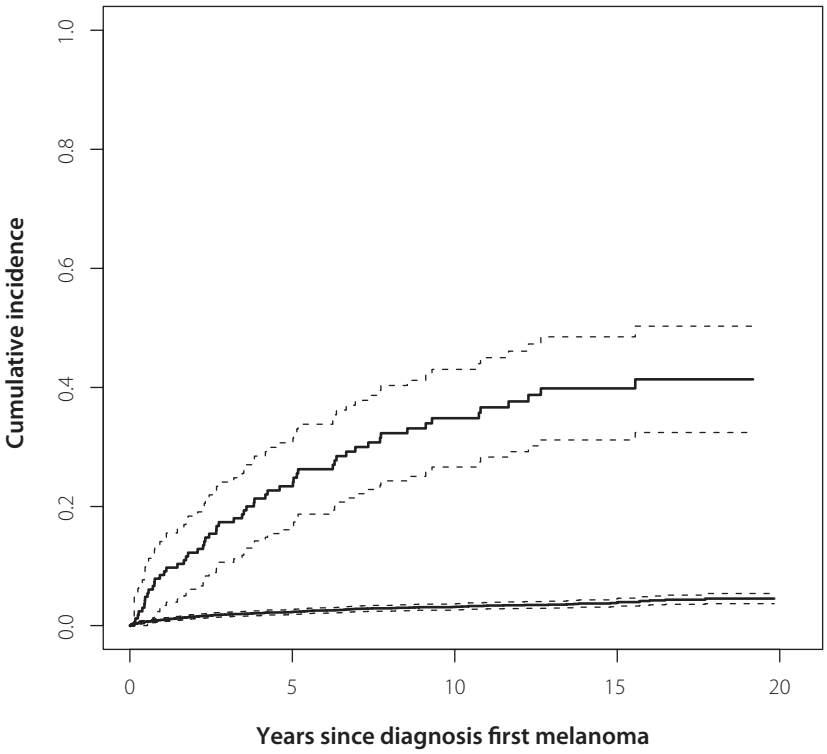
(of the first melanoma) was significantly younger in patients with p16-Leiden (mean: 39.0 years) compared with the control patients (mean: 54.3 years). The mean age difference was 15.3 years (95% confidence interval [CI] 13.3-17.3). In both populations women had their first melanoma at a slightly younger age than men, but the difference was only statistically significant in the control population (control: P <.001; p16-Leiden: P = .189).

Multiple primary melanomas

Patients with p16-Leiden were followed up for a median of 10.4 years (range 0-35.7), and control patients for 5.5 years (range 0-36.0). MPM developed in 40.7% of the patients with p16-Leiden and 3.6% of the control patients. Patients with p16-Leiden and MPM had more primary CMM per patient than control patients with MPM (median: 3.0 [range 2-19] vs 2.0 [range 2-10]).

The estimated 5-year cumulative incidence of a second primary tumor was 23.4% for patients with p16-Leiden and 2.3% for control patients (Table I). Patients with p16-Leiden were at a considerably higher risk of a second CMM than control patients (Fig 1). The HR was dependent on the age of diagnosis of the first melanoma, with a HR of 15.8 (95% CI 11.0-22.7, P < .001) for age younger than 40 years, and a HR of 7.5 (95% CI 4.5-12.7, P < .001) for age older than 40 years. These HRs were significantly different (P value for p16-Leiden status by age group interaction = .016).

Figure 1 Cumulative incidences of second melanomas with 95% confidence intervals in the p16-Leiden (upper line) and control (lower line) population



Risk factors for multiple melanomas

For patients with p16-Leiden, diagnosis of a melanoma before age 40 years was a significant risk factor for the development of a second CMM (HR 1.9, 95% CI 1.2-3.2, P = .011). For control patients localization of the first melanoma in the head and neck region was a statistically significant risk factor for a second CMM (HR 1.61, 95% CI 1.04-2.50, P = .032, "lower extremities" as reference category).

Body site concordance

The overall concordance of body site of the first and second melanoma was 36.1% for patients with p16-Leiden MPM (κ statistics 0.08, SE = 0.071, P = .250) and 52.5% for control patients with MPM (k statistics 0.36, SE = 0.041, P < .001). The difference in body site

concordance between the two populations was statistically significant (P < .001). In the control population concordance was dominated by melanomas in the head and neck region (73.7%) (Table 2).

Table 2 Concordance of body site for first and second primary cutaneous melanoma in patients with multiple melanoma

First primary ↓	Second primary									
	p16-Leiden MPM-patients n (%)					Control MPM-patients n (%)				
	H&N	T	UE	LE	Total	H&N	T	UE	LE	Total
H&N	1 (14%)	5 (71%)	1 (14%)	0 (0%)	7	56 (74%)	6 (8%)	6 (8%)	8 (11%)	76
T	5 (17%)	16 (55%)	5 (17%)	3 (10%)	29	7 (9%)	36 (47%)	19 (25%)	14 (18%)	76
UE	1 (11%)	3 (33%)	1 (11%)	4 (44%)	9	7 (15%)	17 (37%)	15 (33%)	7 (15%)	46
LE	3 (11%)	10 (37%)	6 (22%)	8 (30%)	27	9 (14%)	15 (23%)	10 (15%)	31 (48%)	65
Total	10 (14%)	34 (47%)	13 (18%)	15 (21%)	72	79 (30%)	74 (28%)	50 (19%)	60 (23%)	263

MPM, Multiple Primary Melanomas; H&N, Head and neck; T, Trunk; UE, Upper Extremities; LE, Lower Extremities

Tumor body site and histologic type

A multivariate analysis was performed to investigate differences between p16-Leiden cases and controls concerning tumor type and tumor localization, while adjusting for age of diagnosis, gender, incidence year, and invasiveness. A statistically significant difference in tumor type distribution was found. Patients with p16-Leiden had a smaller proportion of NM (odds ratio 0.38) and LMM/LM (odds ratio 0.20) than control patients (Table 3). There was no difference in tumor localization between patients with p16-Leiden and control patients.

Age at diagnosis related to body site

Patients with p16-Leiden developed melanomas on the head and neck and lower extremities at a younger age than melanomas on the trunk and upper extremities (Table 4). In the control patients head and neck tumors were diagnosed at an older age than tumors on all other body sites. The difference between age at diagnosis by body site pattern of patients with p16-Leiden and control patients was statistically significant (P < .001).

Table 3 Multivariate analysis for characteristics associated with p16-Leiden families

Covariate	OR (95% CI)	p-value
Age	0.96 (0.95 – 0.97)	< 0.001
Gender		
Male	1	
Female	0.70 (0.43 – 1.13)	0.14
Incidence year	0. 91 (0.89 – 0.94)	< 0.001
Invasiveness		
In situ	1	
Invasive	0.74 (0.54 – 1.01)	0.067
Tumor type		
SSM/Mis	1	
NM	0.38 0.25 – 0.57)	< 0.001
LMM/LM	0.20 (0.10 – 0.40)	< 0.001
ALM/ALMis	0.96 (0.38 – 2.44)	0.93
Localization		
Lower extremities	1	
Head/neck	1.35 (0.84 – 2.16)	0.22
Trunk	1.07 (0.79 – 1.43)	0.68
Upper extremities	1.07 (0.77 – 1.49)	0.67

OR, Odds ratio; 95% CI, 95% confidence interval; SSM/Mis, Superficial Spreading Melanoma or Melanoma in situ; NM, Nodular Melanoma; LMM/LM, Lentigo Maligna Melanoma or Lentigo Maligna; ALM/ALMis, Acrolentiginous Melanoma or Acrolentiginous Melanoma in situ

Control patients are the reference population.

Table 4 mean age at diagnosis for different tumor localizations in p16-Leiden and control patients

	p16-Leiden patients		**Control patients**	
Localization	Age* (SD)	n	Age* (SD)	n
Trunk	41.5 yrs (11.8 yrs)	72	50.0 yrs (15.5 yrs)	2495
Head/neck	36.1 yrs (14.6 yrs)	24	65.4 yrs (16.8 yrs)	1447
Upper extremities	41.6 yrs (13.9 yrs)	27	55.0 yrs (17.0 yrs)	1379
Lower extremities	36.3 yrs (13.7 yrs)	57	51.4 yrs (17.4 yrs)	2086
Unspecified	25.3 yrs (14.5 yrs)	2	52.4 yrs (16.6 yrs)	105
Total	39.0 yrs (13.3 yrs)	182	54.3 yrs (17.5 yrs)	7512

*For multiple melanoma patients the age at diagnosis of the first melanoma was taken. SD, standard deviation

Because patients with p16-Leiden had considerably less LMM/LM, which are known to be diagnosed at an older age and frequently occur in the head and neck region, we performed a subanalysis in which we excluded all LMM/LM. The difference in age by body site patterns remained highly statistically significant in this analysis ($P < .001$).

Discussion

We compared the clinical and histologic characteristics of 182 patients with 429 CMM from families with a germline mutation in CDKN2A, with a large control population from the Leiden population-based cancer registry (7512 patients with 7842 CMM). Patients with p16-Leiden melanoma had a younger age of onset and a highly increased risk of MPM, which was highest for patients who had their first melanoma before the age of 40 years. In contrast to control patients with MPM no body site concordance was found for first and second melanomas in patients with p16-Leiden MPM. Patients with p16-Leiden had a higher proportion of SSM/Mis and less NM and LMM/LM. Furthermore, a different age (at diagnosis) by body site pattern was found in the two populations.

The patients with p16-Leiden had a considerably younger age of onset of 39 years. Other studies reported a comparable age at diagnosis for CDKN2A mutation carriers, ranging from 36.3 to 43.3 years.[7-9]

With regard to the occurrence of MPM in patients with p16-Leiden our findings are in accordance with earlier reports, and contain some new observations. Like previous studies on CDKN2A-mutated families, we found a very high proportion of patients with MPM (40.7%) in the p16-Leiden population. It was strikingly higher than the 18.6%[8] and 25.6%[6] of patients with MPM reported in two other studies. The results are difficult to compare, however, because the duration of follow-up was not recorded in these studies. We estimated the 5-year cumulative incidence of second melanomas to be 23.4% in the p16-Leiden population, and 2.3% in the control population, which was similar to the 1.5% to 3.4% reported in other population-based studies.[14-16] In a clinic-based study, Ferrone et al[17] found a 5-year risk of 11.4%. This relatively high risk may have been because of the fact that their study was performed in a tertiary cancer center. Diagnosis of the first melanoma at a young age (<40 years) was associated with an almost doubled risk of MPM in the p16-Leiden population.

Unlike patients with p16-Leiden MPM, there was a statistically significant association between the body site of the first and second melanoma in control patients with MPM. In the control population overall body site concordance was 53%, which was similar to the 48% to 55% reported in earlier studies in comparable populations.[17-19] Like Giles et al,[18] we found concordance to be highest for tumors located in the head and neck area. The

absence of body site concordance in patients with p16-Leiden melanoma, in addition to the highly increased risk of MPM, underlines the importance of frequent and lifelong total-body skin examinations for these patients.

Patients with p16-Leiden had proportionally less NM and LMM/LM, but more SSM/Mis than control patients. There are earlier reports of a decreased proportion of NM in familial melanoma.[20] LMM/LM are diagnosed in extensively sun-damaged skin, usually in elderly people. Because patients with p16-Leiden had a much younger age of onset, less LMM/LM were expected in this patient population. But even though we adjusted for age, the difference remained strongly significant. An increased proportion of superficial spreading type melanomas in familial melanoma has been reported before.[21] It has been suggested that this is because a relatively large proportion of melanomas in patients with familial melanoma arises from (dysplastic) nevi as familial melanoma is associated with increased numbers of (dysplastic) nevi. Melanomas that are associated with nevi are usually of the superficial spreading type.[22] In a recent study Nagore et al[23] observed no acral melanomas among 41 familial melanoma cases. In our study the proportion of ALM/ALM in situ melanomas was similar in patients with p16-Leiden and control patients. The absence reported by Nagore et al[23] may be a result of small sample size in their study.

In accordance with previous studies,[24,25] we found that in the control population, head and neck melanomas were diagnosed at a considerably older age than melanomas on other body sites. Interestingly in the p16-Leiden population head and neck tumors were diagnosed at a younger age than tumors located on the trunk and upper extremities, even after exclusion of LMM/LM from the analysis.

Our findings in the p16-Leiden population are in accordance with the inherited increased susceptibility to melanoma that CDKN2A germline mutations are associated with, including the: (1) young age of onset; (2) high risk of MPM; and (3) absence of body site concordance of the first and second melanoma in patients with MPM. Besides this, our results also suggest that differences exist between the pathogen„esis of melanomas in patients with p16-Leiden and control patients. Several studies have brought forward that melanoma is a heterogeneous
disease.[24,26-28] Whiteman et al[27,28] proposed two distinct etiologic pathways, one associated with increased numbers of nevi, intermittent sun exposure, younger age at diagnosis, and location on the trunk (nevus pathway). The second pathway is associated with chronic sun exposure, fewer nevi, older age at diagnosis, and location in the head and neck region (ultraviolet B pathway). Our results in the control population are in accordance with this theory, as both pathways can be distinguished in this population. First of all, age at diagnosis was considerably higher for tumors in the head and neck region compared with tumors on the trunk, which is in support of the theory of a different origin for these two

body sites. Secondly, we found a statistically significant body site concordance in patients with MPM, with a high concordance for head and neck tumors. This clustering suggests a localized increased melanoma risk, which is in agreement with the divergent pathway theory. In the p16-Leiden population, the high proportion of superficial spreading type melanomas and lack of lentiginous melanomas suggest that melanomas in these patients develop predominantly through the nevus pathway. The young age at diagnosis of melanomas located in the head and neck region is difficult to interpret, however. Relatively high ultraviolet exposure at this body site might play a roll (synergy of the nevus and ultraviolet B pathways).

The ascertainment of patients with p16-Leiden was family based, which is a possible source of bias. Familial melanoma with a young age of onset or MPM are more likely to be identified and genetically tested. The differences between the p16-Leiden population and control population would probably have been smaller if patients with p16-Leiden had been ascertained from a patient population unselected for family history. Such a design has serious drawbacks, however, given the low prevalence of CDKN2A mutations in patients with general melanoma (0.2%-2.0%).[29,30] Moreover, we consider our results to be representative for patients from proven p16-Leiden families, as they present in clinic in daily practice.

In summary, we have verified and substantiated several characteristics of patients with familial melanoma and a CDKN2A mutation in a large case-control study and we have reported a number of new findings. Our findings are in concordance with the so-called divergent pathways hypotheses: familial melanomas tend to follow the nevus pathway. More studies are necessary to determine whether our results apply to founder populations with other CDKN2A mutations as well.

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