

INCREASED RISK OF PANCREATIC CANCER IN MELANOMA-PRONE KINDREDS WITH *p16^{INK4}* MUTATIONS

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Abstract Background. A gene on chromosome 9p, *p16^{INK4}*, has been implicated in the pathogenesis of cutaneous malignant melanoma in 19 melanoma-prone families. In 10 of these kindreds mutations that impaired the function of the *p16^{INK4}* protein (*p16M* alleles) cosegregated with the disease. By contrast, in the other nine kindreds the mutation did not alter the function of *p16^{INK4}* (*p16W* alleles). We looked for differences in clinical and genetic epidemiologic features in these two groups of families.

Methods. We compared the median ages at diagnosis of melanoma, number of melanomas, thickness of the tumors, and number of nevi in the kindreds. We estimated prospectively the risks of melanoma or other cancers in families followed for 6 to 18 years and the risks of other cancers since 1925 (the entire period) by comparing the number of cancer cases observed with the number expected.

Results. The risk of invasive melanoma was increased

by a factor of 75 in kindreds with *p16M* alleles and a factor of 38 in kindreds with *p16W* alleles. Although this difference was not significant ($P=0.14$), there was a striking difference in the risk of other tumors. In kindreds with *p16M* alleles, the risk of pancreatic cancer was increased by a factor of 13 in the prospective period (2 cases observed, 0.15 expected; standardized incidence ratio, 13.1; 95 percent confidence interval, 1.5 to 47.4) and by a factor of 22 in the entire period (7 cases observed, 0.32 expected; standardized incidence ratio, 21.8; 95 percent confidence interval, 8.7 to 44.8). In contrast, we found no cases of pancreatic cancer in kindreds with *p16W* alleles.

Conclusions. The development of pancreatic cancer in kindreds prone to melanoma may require a *p16M* mutation. Genetic factors, such as the kind of mutation found in *p16^{INK4}*, may explain the inconsistent occurrence of other cancers in these kindreds. (N Engl J Med 1995; 333:970-4.)

A LOW-MOLECULAR-WEIGHT protein, *p16^{INK4}*, inhibits the activity of the cyclin D1–cyclin-dependent kinase 4 complex.¹ This complex phosphorylates the retinoblastoma protein, allowing the cell to pass through the G1 cell-cycle checkpoint. Thus, *p16^{INK4}* negatively regulates cell growth by arresting cells at G1. The inactivation of *p16^{INK4}* by deletion or mutation of the gene could lead to unchecked cell growth, suggesting that *p16^{INK4}* may be a tumor-suppressor gene.

The *p16^{INK4}* gene (also known as *MTS1*) was localized to chromosome 9p21,^{2,3} a region that has been implicated in melanoma by studies of linkage, cytogenetics, and loss of heterozygosity.⁴⁻⁹ The findings of germ-line *p16^{INK4}* mutations in some American, European, and Australian melanoma-prone kindreds¹⁰⁻¹³ (and unpublished data) and of somatic mutations in many melanoma cell lines^{2,3} strongly suggest that *p16^{INK4}* is a melanoma tumor-suppressor gene. In addition, functional studies have identified mutations of *p16^{INK4}* that impair the function of its corresponding protein, thus providing a biochemical rationale for the hypothesis that certain *p16^{INK4}* mutations increase the risk of melanoma.¹⁴

In previous work^{10,15} we sought evidence of linkage of

melanoma to chromosome 9p and germ-line *p16^{INK4}* mutations in 19 kindreds with familial melanoma. Nine kindreds¹⁰ plus one that was not described in the reports had *p16^{INK4}* mutations that cosegregated with melanoma and impaired the function of the *p16^{INK4}* protein in in vitro assays (*p16M* alleles, in which *M* designates mutations that impaired function).¹⁴ These mutations included one nonsense (Arg50Ter), one splice-donor-site (IVS2+1), and three missense (Val118Asp, Gly93Trp, and Arg79Pro) mutations¹⁰; one insertion of 24 base pairs; and an uncharacterized mutation that prevents the transcription of *p16^{INK4}* (unpublished data). The other nine kindreds had either no detectable *p16^{INK4}* mutations or a missense mutation (Ile41Thr, Asn63Ser, or Ala140Thr) that did not impair the function of *p16^{INK4}* in in vitro assays (*p16W* alleles, in which *W* designates the wild type or mutations that did not impair function). These latter mutations of *p16^{INK4}* thus have no apparent link to melanomas in the nine kindreds with *p16W* alleles. In the present study, we compared the clinical and genetic epidemiologic characteristics of two groups of kindreds — one with *p16M* and one with *p16W* alleles.

METHODS

Study Population

The study subjects were drawn from 19 families in which there was a history of invasive melanoma in at least two first-degree relatives. The kindreds were divided into two groups: 10 kindreds with *p16^{INK4}* mutations that cosegregated with disease (*p16M* alleles) and 9 kindreds with either no detectable *p16^{INK4}* mutation (5 kindreds) or a mutation that did not impair *p16^{INK4}* function (4 kindreds) (*p16W* alleles). All kindreds had been evaluated previously for evidence of linkage of familial melanoma to chromosome 9p.^{10,15} Thirteen kindreds have also been evaluated for evidence of linkage of familial melanoma to chromosome 1p.^{16,17} These families have been followed prospectively for 6 to 18 years. The ways the families were identified

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varied: some were referred by physicians or other health care professionals, and some were self-referred. All the families were white, were unrelated, and resided in various regions of the United States.

Before each family was evaluated clinically, the diagnosis of melanoma was confirmed by histologic review of tissue from the primary melanoma when possible or, if this was not available, tissue from metastatic-disease sites. If histologic material was no longer available, the diagnosis of melanoma was substantiated by review of local pathology reports, medical records, or death certificates. For each case of invasive melanoma the following information was obtained: the patient's age at diagnosis, the thickness of each tumor (in millimeters), and the total number of invasive tumors. All diagnoses of nonmelanoma cancers were also confirmed by review of histologic materials, local pathology reports, medical records, or death certificates. Clinical examinations to estimate numbers of nevi and confirm the presence or absence of dysplastic nevi and melanoma were performed by a single physician. All pathological material was reviewed by one dermatopathologist.

Statistical Analyses

The mean and median ages at the time melanoma was diagnosed, the number of melanomas, and the thickness of the tumors were estimated for the two groups of families. The medians for these three variables were compared with the use of the nonparametric Mann-Whitney test as implemented in the BMDP3S computer program (nonparametric statistics).¹⁸ Because the number of tumors varied from patient to patient, we calculated an average thickness of the tumors for each patient.

The mean and median number of nevi in the family members we examined were also estimated for the two groups of families. In addition, we compared the distribution of melanoma and dysplastic or clinically atypical nevi, major precursor lesions of melanoma.^{15,17} For this purpose, we compared the relative proportions of melanomas (including melanoma in situ) and dysplastic nevi in the kindreds in the two groups of families.

To estimate the prospective risk of invasive melanoma or other cancers, we used the computer program of Monson¹⁹ to calculate numbers of person-years of observation according to sex, age, and the interval from the date of the first clinical examination to the development of melanoma or other cancer, death, or December 31, 1994. Tumor-incidence rates specific for sex, age, and calendar year were obtained from the Surveillance, Epidemiology, and End Results (SEER) Program and were multiplied by the total number of person-years to estimate the number of cases of melanoma or other cancers expected if this group had had the same risk of cancers as the general population.²⁰ The statistical methods used to estimate risk were based on the assumption that the number of melanomas and other cancers observed followed a Poisson distribution. Tests of significance and confidence intervals for the standardized incidence ratios (the ratio of the number of observed cases to the number expected) were calculated exactly on the basis of a Poisson distribution.²¹ Tests for homogeneity were performed as implemented in the Epihome computer program.²¹

We also analyzed the risk of other cancers (excluding melanoma) for the entire risk period (i.e., the retrospective and prospective periods). For this analysis, we calculated the numbers of person-years of observation according to sex, age, and the interval from the later of two dates — the person's birth date or January 1, 1925 (the first year for which back-estimated SEER rates were available) — to the development of nonmelanoma cancer, death, or December 31, 1994. These results were similar to those derived from the Connecticut Tumor Registry.

RESULTS

Overall, the median age at which melanoma was diagnosed was 34 years (Table 1), which is substantially earlier than in the general population.²² The age at di-

Table 1. Clinical Characteristics of 19 Melanoma-Prone Kindreds.

CHARACTERISTIC	NO. OF SUBJECTS	MEAN ± SD	MEDIAN	RANGE	P VALUE*
Age at diagnosis of melanoma (yr)		35.72±13.8	34		0.05
Kindreds with <i>p16W</i> alleles	40	39.22±14.4	36.5		
Kindreds with <i>p16M</i> alleles	56	33.21±12.9	30.5		
Tumor thickness (mm)		1.02±1.14	0.80		0.74
Kindreds with <i>p16W</i> alleles	40	1.18±1.53	0.80		
Kindreds with <i>p16M</i> alleles	56	0.91±0.75	0.79		
No. of melanomas		1.98±2.15	1.0		0.09
Kindreds with <i>p16W</i> alleles	40	1.68±1.80	1.0		
Kindreds with <i>p16M</i> alleles	56	2.20±2.36	1.0		
No. of nevi†	313	63.2±70.3	37	0–541	0.31
Kindreds with <i>p16W</i> alleles	114	70.9±85.4	37	2–541	
Kindreds with <i>p16M</i> alleles	199	58.8±59.7	37	0–413	

*For the differences between the two groups, by the Mann-Whitney test.

†At the time of the clinical examination.

agnosis was marginally lower in the kindreds with *p16M* alleles ($P=0.05$). To determine whether ascertainment bias accounted for this difference between groups, we excluded the index patients (the first two patients with melanoma in each family) from the analysis. The difference in age at diagnosis was no longer significant (27.5 years in the kindreds with *p16M* alleles vs. 35.5 years in the kindreds with *p16W* alleles, $P=0.09$). In both groups, however, the median age at which melanoma was diagnosed was at least 18 years lower than the median age at which melanoma is diagnosed in the white population in the United States (median age, 54 years).²² There were no significant differences between the two groups in the number of melanomas ($P=0.09$) or tumor thickness ($P=0.74$). Exclusion of the index patients did not alter these results.

There was no significant difference in the number of nevi between the two groups of kindreds ($P=0.31$) (Table 1). Adjustment for age had no effect on the results (data not shown). All 19 kindreds had dysplastic nevi in addition to melanoma. We therefore compared the distribution of melanoma and dysplastic nevi in the kindreds. There was no difference in the relative proportion of family members with melanoma between the kindreds with *p16M* alleles and the kindreds with *p16W* alleles (0.60 vs. 0.65, $P=0.54$).

Table 2 shows the prospective risk of melanoma and other cancers in the two groups of kindreds. Cancers were considered according to organ system rather than individual site (for one or fewer cancers) because of the relatively small numbers. The prospective risk of melanoma was increased by a factor of 75 in kindreds with *p16M* alleles (standardized incidence ratio, 74.6; 95 percent confidence interval, 45.6 to 115.2) and a factor of 38 in kindreds with *p16W* alleles (standardized incidence ratio, 38.1; 95 percent confidence interval, 13.9 to 82.9). This difference was not statistically significant ($P=0.14$). However, there was a striking difference in the risk of other tumors between the two groups. The prospective risk of pancreatic cancer was increased by a factor of 13 (standardized incidence ratio, 13.1; 95 percent confidence interval, 1.5 to 47.4) in the kindreds with *p16M* alleles. The two observed cases of pancreatic cancer were in different families. In both cases there was a previous diagnosis of invasive melanoma or melanoma

in situ. There were no cases of pancreatic cancer among the kindreds with *p16W* alleles.

Table 3 shows the risks of other cancers in the two groups of kindreds during the entire risk period. Since families were enrolled in the study on the basis of a history of melanoma, melanomas were excluded from this analysis. However, we would expect the assessment of other cancers to be similar in the prospective and entire (i.e., retrospective plus prospective) risk periods if families were not selected because they had other cancers. Overall, there was no excess of cancer in either group. However, the risk of digestive-system cancers was increased by a factor of 3 in the kindreds with *p16M* alleles because of the excess of pancreatic cancer (standardized incidence ratio, 21.8; 95 percent confidence interval, 8.7 to 44.8) in kindreds with *p16M* alleles. In addition, there was a significant difference in the standardized incidence ratio for pancreatic cancer between the two groups of kindreds ($P=0.02$). The seven cases of pancreatic cancer occurred in four different families with *p16M* alleles, three of which had two cases of pancreatic cancer each (Table 4). Three patients had also had invasive melanoma or melanoma in situ. One of the patients with pancreatic cancer had a *p16^{INK4}* mutation; two were obligate carriers of a mutation. The *p16^{INK4}* status of the other four patients could not be determined. There was no relation between specific *p16^{INK4}* mutations and the risk of pancreatic cancer; each kindred with pancreatic cancer had a different *p16^{INK4}* mutation.¹⁰ Thus, overall, 4 of the 10 kindreds with *p16M* alleles had at least one family member each with pancreatic cancer, as compared with none of the 9 kindreds with *p16W* alleles.

DISCUSSION

We evaluated 10 melanoma-prone kindreds with *p16M* alleles and 9 with *p16W* alleles to determine

Table 3. Overall Risk of Nonmelanoma Cancer in Bloodline Members of Melanoma-Prone Kindreds with *p16M* or *p16W* Alleles.*

TYPE OF TUMOR	KINDREDS WITH <i>p16M</i> ALLELES (PERSON-YR, 10,940.5)†				KINDREDS WITH <i>p16W</i> ALLELES (PERSON-YR, 8255.0)†			
	OBS	EXP	OBS/EXP	95% CI	OBS	EXP	OBS/EXP	95% CI
	no. of cases				no. of cases			
All cancers	19	15.21	1.2	0.8–2.0	12	12.79	0.9	0.5–1.6
Digestive system	9	2.73	3.3	1.5–6.3	2	2.25	0.9	0.1–3.2
Pancreas	7	0.32	21.8	8.7–44.8	0	0.27		0.0–13.8
Respiratory system	5	2.43	2.1	0.7–4.8	4	2.08	1.9	0.5–4.9
Lung	4	2.14	1.9	0.5–4.8	4	1.84	2.2	0.6–5.6
Female breast	2	2.28	0.9	0.1–3.2	1	2.13	0.5	0.0–2.6
Prostate	1	1.11	0.9	0.0–5.0	3	0.85	3.6	0.7–10.4
Urinary tract	1	1.04	1.0	0.0–5.4	1	0.80	1.2	0.0–6.9
Lymphatic or hematopoietic	1	1.56	0.6	0.0–3.6	1	1.22	0.8	0.0–4.5

*Obs denotes observed, exp expected, and CI confidence interval.

†The number of person-years of follow-up is given for each group.

whether there were differences in clinical features and the risk of various cancers. Although the difference was not significant, kindreds with *p16M* alleles had a lower median age when melanoma was diagnosed than kindreds with *p16W* alleles. In both groups, however, the median age at diagnosis was substantially lower than that in the general U.S. population. There were no significant differences in the number of melanomas or in tumor thickness in the two groups, nor were there differences in the number of nevi or in the proportions of melanoma and dysplastic nevi. The prospective risk of melanoma was increased by a factor of 75 in kindreds with *p16M* alleles and a factor of 38 in kindreds with *p16W* alleles — a difference that was not significant. By contrast, the risk of pancreatic cancer was significantly increased only in kindreds with *p16M* alleles.

Several researchers have investigated whether familial susceptibility to melanoma increases the risk of other cancers independently of other known familial cancer syndromes (e.g., Li–Fraumeni syndrome²³). The present 19 kindreds were part of a prospective follow-up study of 23 melanoma-prone families in the United States that found no significant excess of cancers in these families other than melanoma.²⁴ Kopf et al.²⁵ also observed that patients with familial melanoma had fewer cancers of other types than those with sporadic melanoma, and Swerdlow et al.,²⁶ who examined the risk of second primary cancer in all patients with cutaneous melanoma (total, 12,460) in Denmark from 1943 to 1989 (follow-up, 88,667 person-years), found no increased risk of pancreatic cancer in these patients.

In contrast, Lynch et al.²⁷ described a large kindred with familial atypical multiple-mole melanoma with an increased risk of several other cancers, including intraocular melanoma and carcinoma of the lung, skin, larynx, and breast. Since this study was limited to a single kindred, it is possible that the reported excess may have represented chance

Table 2. Prospective Risk of Cancer in Bloodline Members of Melanoma-Prone Kindreds with *p16M* or *p16W* Alleles.*

TYPE OF TUMOR	KINDREDS WITH <i>p16M</i> ALLELES (PERSON-YR, 2237.8)†				KINDREDS WITH <i>p16W</i> ALLELES (PERSON-YR, 1437.5)†			
	OBS	EXP	OBS/EXP	95% CI	OBS	EXP	OBS/EXP	95% CI
	no. of cases				no. of cases			
All cancers	27	6.88	3.9	2.6–5.7	9	4.01	2.2	1.02–4.3
Digestive system	4	1.31	3.0	0.8–7.8	0	0.76		0.0–4.8
Pancreas	2	0.15	13.1	1.5–47.4	0	0.09		0.0–41.7
Respiratory system	1	1.08	0.9	0.0–5.1	2	0.67	3.0	0.3–10.7
Lung	1	1.02	1.0	0.0–5.5	2	0.60	3.3	0.4–12.0
Female breast	1	1.02	1.0	0.0–5.5	0	0.66		0.0–5.6
Prostate	0	0.69		0.0–5.3	1	0.38	2.7	0.0–14.8
Urinary tract	0	0.48		0.0–7.7	0	0.26		0.0–14.2
Melanoma	20	0.27	74.6	45.6–115.2	6	0.16	38.1	13.9–82.9
Lymphatic or hematopoietic	1	0.59	1.7	0.0–9.5	0	0.34		0.0–10.8

*Obs denotes observed, exp expected, and CI confidence interval.

†The number of person-years of follow-up is given for each group.

Table 4. *p16^{INK4}* Mutations in Melanoma-Prone Kindreds with Pancreatic Cancer According to the Number of Bloodline Relatives with Melanoma, Pancreatic Cancer, or Both.

FAMILY No.	MUTATION	no. of bloodline relatives		
		PANCREATIC CANCER ONLY	MELANOMA AND PANCREATIC CANCER*	MELANOMA ONLY*
479	Val118Asp†	1	1	5
481	Gly93Trp	2	0	3
873	Arg79Pro	0	1	9
2884	IVS2+1‡	1§	1	2

*Includes both invasive melanoma and melanoma in situ.

†Missense mutation resulting in a codon change from valine to aspartic acid in codon 118.

‡Splice-donor-site mutation.

§This patient was said to have had melanoma on the basis of history. No confirmation of the diagnosis was possible.

cosegregation of rare cancers rather than an increased risk of other cancers in melanoma-prone families. Subsequently, Bergman et al.²⁸ observed an increased frequency of gastrointestinal tract neoplasms, particularly carcinoma of the pancreas, in some Dutch melanoma-prone kindreds. Our finding that the risk of pancreatic cancer was increased only in melanoma-prone kindreds with *p16M* alleles may partly explain these variations in familial melanoma.

The finding of an increased risk of pancreatic cancer was based on only two prospective cases and seven total cases and thus requires corroboration before this information can be applied in a clinical setting. However, the finding of multiple cases of pancreatic cancer in three of the four kindreds with pancreatic cancer suggests that health care professionals should carefully check the family history of melanoma-prone kindreds.

Abnormalities of *p16^{INK4}* have been linked directly to pancreatic adenocarcinoma. Caldas et al.²⁹ found frequent somatic mutations and homozygous deletions of the *p16^{INK4}* gene in pancreatic carcinomas. They observed allelic deletions of chromosome 9p21-p22, the region that harbors *p16^{INK4}*, in 85 percent (22 of 26) of informative tumors. In addition, Caldas et al. examined 37 pancreatic carcinomas (27 xenografts and 10 cell lines) and found homozygous deletions of the *p16^{INK4}* gene in 15 (41 percent) and sequence changes in 14 (38 percent).²⁹ These results suggest that abnormal regulation of cyclin-dependent kinases may have an important role in the biology of pancreatic carcinoma.

Mutations in *p16^{INK4}* have been detected in only one third to one half of the melanoma-prone kindreds examined to date¹⁰⁻¹³ (and unpublished data). Nevertheless, in a small number of kindreds without detectable *p16^{INK4}* mutations there was strong evidence of linkage of familial melanoma to chromosome 9p21-p22.^{9-13,15,30,31} These kindreds may have undetected *p16^{INK4}* mutations, or there may be another gene relevant to melanoma in this chromosomal region. It is also possible that the melanoma in families without *p16^{INK4}* mutations may represent chance aggregations of small clusters of cases. Although two of the kindreds with *p16W* alleles had only two family members with melanoma, the average

number of persons with melanoma (invasive and in situ) was similar in the kindreds with *p16M* and *p16W* alleles (5.8 vs. 5.2, respectively), suggesting that the majority of cases of melanoma in kindreds with *p16W* alleles do not result from chance aggregations of small clusters of cases.

One kindred (Family 373) had a missense mutation (Asn63Ser) that cosegregated with the combined trait of melanoma and dysplastic nevi.¹⁰ However, functional studies in in vitro assays¹⁴ suggested that this mutation had little ability to impair the function of p16^{INK4}. Thus, this kindred was included in the group with *p16W* alleles. Neither the exclusion of this kindred from the group with *p16W* alleles nor its inclusion in the group with *p16M* alleles had much effect on the results (data not shown).

In summary, previous examinations of familial melanoma have inconsistently shown relations between melanoma and pancreatic cancer. This inconsistency may be due to pancreatic cancer's being associated only with familial melanoma involving *p16M* mutations. Thus, information about genetic factors, such as *p16^{INK4}*, may help explain the inconsistent occurrence of other cancers in kindreds with familial melanoma.

We are indebted to the participating families, whose generosity and cooperation have made this study possible, and to Kevin Meagher for analytical support.

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