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Germline *CDKN2A* Mutation Status and Survival in Familial Melanoma Cases

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Abstract

Background: Germline mutations in *CDKN2A* have been associated with increased risk of melanoma and tobacco-related cancers in respiratory and upper digestive tissues. In *CDKN2A* wild-type (wt) melanoma families, other known high-risk, melanoma-predisposing mutations are rare, and no increased risk has been observed for nonskin cancers in this group. This study is the first to compare survival in germline *CDKN2A* mutated (mut) and nonmutated melanoma cases.

Methods: Melanoma-prone families participating in this study were identified through a nationwide predictive program starting in 1987. Information on cancer diagnoses (types, stages, and dates) and deaths (causes and dates) were obtained through the Swedish Cancer Registry and Cause of Death Registry. Kaplan Meier and Cox proportional hazards regression models were used to assess survival in $CDKN2A^{mut}$ (n = 96) and $CDKN2A^{wt}$ (n = 377) familial melanoma cases and in matched sporadic melanoma cases (n = 1042). All statistical tests were two-sided.

Results: When comparing $CDKN2A^{mut}$ and $CDKN2A^{wt}$ melanoma cases, after adjusting for age, sex, and T classification, $CDKN2A^{mut}$ had worse survival than melanoma (hazard ratio [HR] = 2.50, 95% confidence interval [CI] = 1.49 to 4.21) and than nonmelanoma cancers (HR = 7.77, 95% CI = 3.65 to 16.51). Compared with matched sporadic cases, $CDKN2A^{mut}$ cases had statistically significantly worse survival from both melanoma and nonmelanoma cancers while no differences in survival were seen in $CDKN2A^{wt}$ compared with sporadic cases.

Conclusions: $CDKN2A^{mut}$ cases had statistically significantly worse survival than nonmelanoma cancers and, intriguingly, also from melanoma, compared with melanoma cases with no *CDKN2A* mutations. Further studies are required to elucidate possible mechanisms behind increased carcinogen susceptibility and the more aggressive melanoma phenotype in *CDKN2A* mutation carriers.

Germline mutations in the tumor suppressor gene *CDKN2A* are among the strongest known inherited genetic risk factors for cutaneous melanoma. Carriers have a risk of melanoma that is greater than 65-fold increased, compared with the normal population, and a lifetime penetrance for melanoma of 60% to 90% (1–3). *CDKN2A* mutation penetrance is associated with population incidence rates of melanoma, and the same factors that affect population incidence of melanoma, such as UV exposure, pigmentation traits, and risk-modifying genes, appear to mediate *CDKN2A* penetrance (2,4). In carriers of some *CDKN2A* mutations, increased risks have also been reported for nonmelanocytic cancers, in particular pancreatic, lung, and head and neck cancers (1,3,5–9), which occur more prominently in carriers who smoke tobacco (1,9). Collectively, these findings indicate that the development of tumors in *CDKN2A* mutation carriers is also dependent on interactions between environmental and host factors other than the germline mutation. High-risk melanoma-associated germline mutations in other genes than *CDKN2A* have yet only been identified in very few melanoma families. We have previously reported that in a cohort of 1168 members of melanoma-prone families with *CDKN2A* wild-type (wt) status,

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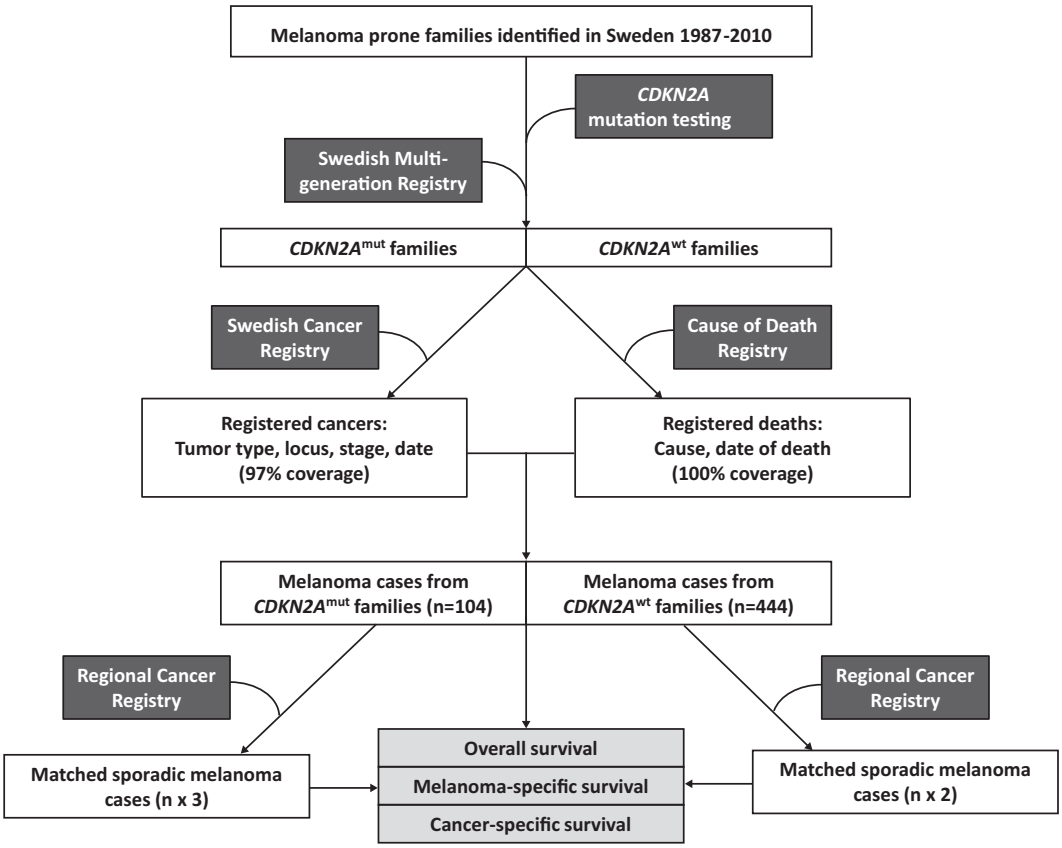

Figure 1. Flowchart to describe study participants and register linkages. mut = mutated, wt = wild-type.

there was a statistically significantly increased risk of melanomas and squamous cell skin cancers but no increased risk of nonskin cancers (10). Furthermore, the allele frequencies of red hair color variants of the pigmentation gene *MC1R* were observed to be highest in *CDKN2A* wt melanoma cases, intermediate in sporadic melanoma cases, and lowest in healthy control subjects, indicating a gene dosage effect (10,11). In the Swedish *CDKN2A* wt melanoma families, it is likely that many members harbor skin cancer–associated gene variants and pigmentation traits that, in combination with UV exposure, cause a familial sensitivity to developing both melanoma and nonmelanoma skin cancers, and probably only relatively few families have high-risk cancer-associated mutations in tumor suppressor genes or oncogenes (10).

Several studies have compared patient- and tumor-specific factors among germline *CDKN2A* mutation carriers and noncarriers. In summary, the only consistent finding has been that *CDKN2A* mutation carriers are younger at melanoma diagnosis whereas findings on differences in tumor-specific features such as body site of the primary tumor, invasiveness, thickness, and histological type have been statistically nonsignificant or divergent (12–16). There have been indications that primary melanomas from *CDKN2A* mutation carriers are less advanced at diagnosis; however, in most of the studies, control subjects have consisted of sporadic melanoma cases (12–14). This is of importance because members of melanoma families are normally enrolled in dermatologic follow-up programs aimed at diagnosing melanomas at earlier or premalignant stages while sporadic melanoma cases have usually not participated in any such programs. Also, it is not specified in the reviewed studies whether the first melanoma diagnosed or subsequent melanomas were used for comparative analysis. This is also important because multiple melanomas are common among familial melanoma cases and the first melanoma is usually the thickest (17–20). After the first melanoma diagnosis, awareness increases, resulting in subsequent melanomas being thinner and less invasive. The presumption that melanoma tumors of *CDKN2A* mutation carriers have more favorable prognostic features has led to speculations that mutation carriers might have similar or even more beneficial outcomes from their melanomas, but research addressing this specific question has until now been entirely lacking. The aim of this study was to evaluate survival from all causes, from melanoma alone, and from nonmelanoma cancers in familial melanoma cases with mutations in the *CDKN2A* gene, compared with familial and sporadic cases without such mutations.

Methods

Participants and Register Linkages

In 1987, the Swedish Melanoma Study Group initiated a national program to identify kindreds with familial cutaneous malignant melanoma and to provide members of these families the possibility to participate in a preventive program (17). Melanoma families were defined as kindreds with at least two blood relatives (first-, second-, or third degree-relatives) with verified melanomas (in situ or invasive). Melanoma families were identified through questioning of newly diagnosed melanoma patients regarding relatives with melanoma. Melanoma tumors in relatives were verified by pathology and/or clinical records. In this survival study, families where at least one familial melanoma case had been screened for germline mutations in the *CDKN2A* gene were included. In the flowchart in Figure 1, an overview of study

Table 1. Characteristics of cases and tumors in *CDKN2A*^{mut} and *CDKN2A*^{wt} melanoma families

Characteristics	*CDKN2A*^{mut} (n = 104)	*CDKN2A*^{wt} (n = 444)	*P**
Families and melanomas diagnosed			
Year when family identified, median (range)	95 (1993–2010)	97 (1993–2010)	.23
Number of families, No.	31	238	
Numbers of melanomas, No.	201	536	
Melanoma diagnoses per family, median (range)	6 (2–12)	2 (2–19)	<.001
CDKN2A mutation status, No. (%)			
Confirmed	80 (76.9)	319 (71.8)	.29
Assumed	24 (23.1)	125 (28.2)	
Sex, No. (%)			
Female	61 (58.7)	231 (52.0)	.22
Male	43 (41.3)	213 (48.0)	
Age, median (range), y			
Age at diagnosis of first melanoma	40 (16–86)	50 (14–92)	.002
Multiple primary melanoma, No. (%)			
Yes	42 (40.4)	65 (14.6)	<.001
No	62 (59.6)	379 (85.4)	
Numbers of melanomas/individual, No. (%)			
1	62 (59.6)	379 (85.4)	<.001
2	24 (23.1)	49 (11.0)	
3	6 (5.8)	14 (3.1)	
>3	12 (11.5)	2 (0.5)	
T classification of subsequent melanomas diagnosed, No. (%)			
T classification first melanoma ≥ subsequent melanoma(s)	27 (93.1)	48 (94.1)	.86
T classification first melanoma < subsequent melanoma(s)	2 (6.9)	3 (5.9)	
Unknown T classification of first or any subsequent melanoma	13	14	
Site of melanoma (all diagnosed), No. (%)			
Head/neck	16 (8.4)	61 (11.8)	.08
Trunk	82 (43.2)	248 (48.0)	
Upper extremities	41 (21.6)	74 (14.3)	
Lower extremities	51 (26.8)	134 (25.9)	
Unknown	11	19	
Invasiveness of all diagnosed melanoma, No. (%)			
In situ	45 (22.4)	123 (23.0)	.87
Invasive	156 (77.6)	413 (77.0)	
Invasiveness of first diagnosed melanoma, No. (%)			
In situ	14 (13.5)	71 (16.0)	.52
Invasive	90 (86.5)	373 (84.0)	
T classification† of first invasive melanoma, No. (%)			
T1	53 (63.1)	194 (57.7)	.46
T2	15 (17.9)	87 (25.8)	
T3	9 (10.7)	28 (8.3)	
T4	7 (8.3)	28 (8.3)	
Unknown	12	40	
Diagnosis of nonmelanoma cancer‡, No. (%)			
Yes	43 (41.3)	120 (27.0)	.004
No	61 (58.7)	324 (73.0)	

*Two-sided *P* values calculated by Chi-Square test, except for age and year, where Student's *t* test was used. *CDKN2A*^{mut} = familial melanoma case with germline *CDKN2A* gene mutation; *CDKN2A*^{wt} = familial melanoma case with no *CDKN2A* mutations (wild-type).
†T classification of the primary tumor according to the 2009 American Joint Committee on Cancer melanoma staging and classification system.
‡Nonmelanoma cancer: all cancer types except melanoma.

participants and register linkages is shown. The national 10-digit personal identity number of each *CDKN2A*-tested familial melanoma index case was linked with the Swedish Multi-Generation Registry, which contains connections between all individuals born after 1931 and their biological parents, who were registered in Sweden after 1960 (21). This allowed identification of familial melanoma cases' first-degree relatives (parents, siblings, and children) and second-degree relatives (grandparents, uncles/aunts, nieces/nephews, half-siblings, and grandchildren). The personal identity numbers of all identified family members were linked with the Swedish Cancer Registry to obtain data on all registered cancers (types, stages, and dates) and with the Cause of Death Registry to obtain data on all deaths (causes and dates). The Swedish Cancer Registry was established in 1958, and reporting to this registry is compulsory for both clinicians and pathologists/cytologists diagnosing a cancer; completeness of the register has been estimated to 97% (22). To identify registered cancer types, ICD7 codes and histology codes (WHO/HS/CANC/24.1) were used; for the T classifications of primary melanoma tumors, the 2009 American Joint Committee on Cancer melanoma staging and classification system was used (23). In the Cause of Death registry, information on all deaths of individuals

Table 2. Causes of death in *CDKN2A*^{mut} and *CDKN2A*^{wt} and sporadic melanoma cases

Deaths	*CDKN2A*^{mut} (n = 104) No. (%)	*CDKN2A*^{wt} (n = 444) No. (%)	Sporadic melanoma (n = 312)* No. (%)	Sporadic melanoma (n = 888)† No. (%)	P‡,§	P‡,‖	P‡,¶
All deceased	47 (45.2)	135 (30.4)	105 (33.7)	294 (33.1)	.004	.03	.28
Non–cancer related	6 (5.8)	56 (12.6)	41 (13.1)	117 (13.2)	.024	.04	.77
Melanoma	23 (22.1)	56 (12.6)	44 (14.1)	127 (14.3)	.01	.05	.71
Nonmelanoma cancer	18 (17.3)	23 (5.2)	20 (6.4)	50 (5.6)	<.001	<.001	.73
Pancreas	8 (7.7)	2 (0.5)	1 (0.3)	3 (0.3)	<.001	<.001	.75
Lung	5 (4.8)	3 (0.7)	1 (0.3)	9 (1.0)	.001	<.001	.38
Breast	2 (1.9)	2 (0.5)	4 (1.3)	7 (0.8)	.11	.26	.50
Gastrointestinal	1 (1.0)	6 (1.4)	2 (0.6)	10 (1.1)	.76	.74	.72
Urinary	1 (1.0)	6 (1.1)	2 (0.6)	9 (1.0)	.75	.74	.58
Head and neck	0 (0)	0 (0)	0 (0)	1 (0.1)	—#	—#	—#
Gynecological	0 (0)	0 (0)	3 (0.9)	2 (0.2)	—#	—#	—#
Other	1 (1.0)	4 (0.9)	7 (2.2)	9 (1.0)	.95	.41	.84

*Sporadic melanoma cases, matched for age, sex, year of diagnosis, and tumor thickness of CDKN2A^{mut} cases. *CDKN2A*^{mut} = familial melanoma case with germline *CDKN2A* gene mutation; *CDKN2A*^{wt} = familial melanoma case with no *CDKN2A* mutations (wild-type).
†Sporadic melanoma cases, matched for age, sex, year of diagnosis, and tumor thickness of CDKN2A^{wt} cases.
‡All *P* values are two-sided and calculated by Chi-Square test.
§*P* value comparing *CDKN2A*^{mut} and *CDKN2A*^{wt} cases.
‖*P* value comparing *CDKN2A*^{mut} and matched sporadic melanoma cases.
¶*P* value comparing *CDKN2A*^{wt} and matched sporadic melanoma cases.
#Noncalculable because of 0 cases.

living in Sweden has been registered since 1961 (24). Only family members who had a registered melanoma diagnosis were included in this survival study. Sporadic melanoma cases, matched for age, sex, T classification, and year of diagnosis, were obtained from the Regional Melanoma Registry of the Stockholm-Gotland health care region. In a previous study on sporadic melanoma cases from this region, a *CDKN2A* mutation was found in only one of 526 cases (0.2%) (11).

CDKN2A Mutation Analyses

Melanoma family members were invited to undergo germline *CDKN2A* mutation analysis for the purpose of study. Procedures used for DNA isolation from peripheral blood mononuclear cells, polymerase chain reaction (PCR) of *CDKN2A* exons, and direct sequencing of PCR products have been described previously (1). Informed consent was obtained before family members underwent mutation analysis, and the study was approved by Research Ethical Review Boards in Lund and Stockholm. Of the 270 identified families, protein-altering mutations have been identified in 31 families, of which 29 carry the Swedish founder mutation in *CDKN2A* (p.Arg112dup), one has an in-frame deletion of 24 bases (p.delAla60_Gly67), and one has an amino acid substitution (p.Pro48Leu). It has previously been reported that these mutations disrupt function of the p16 protein and segregate with melanoma (8,25,26).

Follow-up

Follow-up started at the date when the first invasive melanoma was diagnosed in each case. The *CDKN2A* mutation test has, until now, solely been a research test in Sweden, and all identified melanoma families have been enrolled in the same clinical follow-up program, with regular skin exams and education on skin cancer prevention, irrespective of *CDKN2A* mutation status. Follow-up ended at the date of death, emigration, or census date of December 31, 2011.

Statistical Analysis

Survival from all causes, from melanoma, and from nonmelanoma cancers (all cancer types except melanoma) was studied. For overall survival, all deaths were considered events. For melanoma-specific survival, only deaths from melanoma were counted as events; deaths from other causes were labeled as censored, and follow-up ended on the date of death. In the same way, for nonmelanoma cancer–specific survival, only deaths from nonmelanoma cancers were counted as events, deaths from other causes were labeled as censored, and follow-up ended on the date of death. For survival plots, Kaplan-Meier analyses with log-rank *P* values were computed. Cox proportional hazards regression models, unadjusted and adjusted for age, sex, and T classification of the primary tumor were used to assess survival in *CDKN2A*^{mut} compared with *CDKN2A*^{wt} familial melanoma cases. Proportional hazards hypothesis was verified by investigating Schoenfeld residuals. All statistical tests were two-sided. *P* values under .05 were considered statistically significant. Statistical analyses were performed with StatSoft Statistica software, version 10.

Results

Patient and Tumor Characteristics of Familial Melanoma Cases

The *CDKN2A*^{mut} (n = 104) and *CDKN2A*^{wt} (n = 444) melanoma cases (in situ and invasive) were identified from 31 *CDKN2A*^{mut} and 238 *CDKN2A*^{wt} melanoma families, respectively (Table 1). In the *CDKN2A*^{mut} and *CDKN2A*^{wt} familial melanoma cases, 76.9% and 71.8% had a confirmed mutation status, respectively. Familial melanoma cases lacking mutation data who were assumed to be *CDKN2A* mutation–positive or mutation-negative were first- and second-degree relatives of *CDKN2A*^{mut} or *CDKN2A*^{mut} cases, respectively. There was a somewhat higher percentage of women among *CDKN2A*^{mut} cases compared with *CDKN2A*^{wt} (58.7% vs 52.0%), but this difference was not

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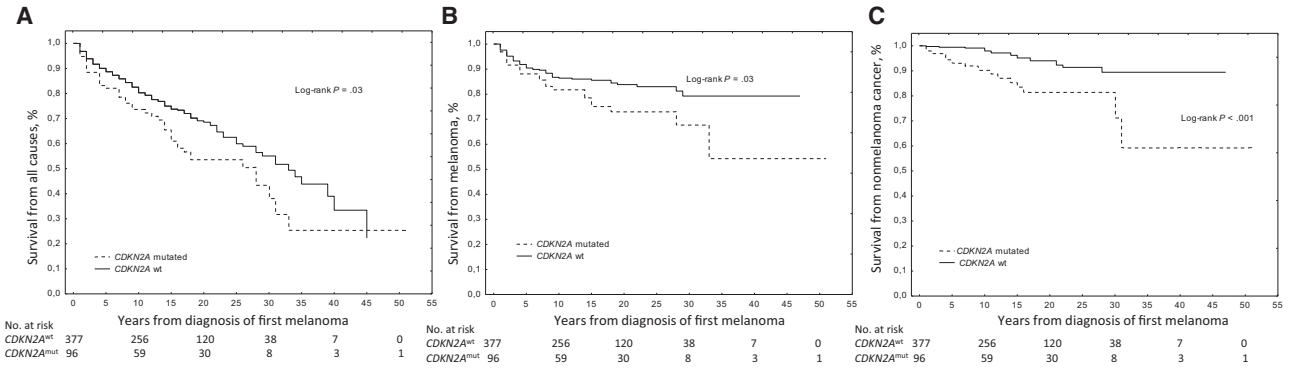

Figure 2. Kaplan-Meier curves for survival from all causes **(A)**, from melanoma **(B)**, and from nonmelanoma cancer **(C)** in *CDKN2A*^{mut} compared with *CDKN2A*^{wt} familial melanoma cases. All statistical tests were two-sided. wt = wild-type.

statistically significant. The *CDKN2A*^{mut} cases were statistically significantly younger than *CDKN2A*^{wt} cases at the diagnosis of their first melanoma (40 years vs 50 years, $P = .002$). The total number of melanoma diagnoses (in situ and invasive) was 201 in the *CDK2NA*^{mut} cases and 536 in the *CDKN2A*^{wt} cases. The median numbers of melanoma diagnoses in each *CDK2NA*^{mut} and *CDKN2A*^{wt} family were six and two, respectively. Multiple primary melanomas were statistically significantly increased in mutation carriers; 40.4% had multiple primary melanomas, and 17.3% had three or more melanomas, compared with 14.6% and 3.6%, respectively, in *CDKN2A*^{wt} melanoma cases. Of the multiple primary melanoma cases, a minority were confirmed to have subsequent melanomas of a higher T classification, 6.9% of the CDKN2A^{mut} cases and 5.9% of *CDKN2A*^{wt} cases. There were no statistically significant differences in the primary tumor site, invasiveness, or T classification of melanomas between *CDKN2A*^{mut} and *CDKN2A*^{wt} melanoma cases. Nonmelanoma cancers were statistically significantly overrepresented among the *CDKN2A*^{mut} compared with the *CDKN2A*^{wt} familial melanoma cases ($P = .004$); 41.3% were diagnosed with other tumors, compared with 27.0% of the *CDKN2A*^{wt} melanoma cases.

Causes of Death Among *CDKN2A*^{mut}, *CDKN2A*^{wt}, and Sporadic Melanoma Cases

Of the *CDKN2A*^{mut} familial melanoma cases (in situ and invasive), 45.2% were deceased at the censor date, compared with 30.4% of the *CDKN2A*^{wt} cases (Table 2). This statistically significant difference in deaths ($P = .004$) from any cause was seen even though non-cancer-related deaths were more frequent in the *CDKN2A*^{wt} cases (5.8% vs 12.6%). The increased frequency of deaths in the *CDKN2A*^{mut} cases was explained by a statistically significantly higher mortality in *CDKN2A*^{mut} cases from both melanoma (22.1% vs 12.6%, $P = .01$) and nonmelanoma cancers (17.3% vs 5.2%, $P < .001$) compared with the *CDKN2A*^{wt} cases. Among the matched sporadic melanoma cases, the numbers and causes of deaths were comparable with those in the *CDKN2A*^{wt} group. In particular, *CDKN2A*^{mut} cases had many deaths from pancreatic and lung cancers; of the 18 nonmelanoma cancer deaths among *CDKN2A*^{mut} cases, 13 (72%) were from pancreatic or lung cancer, compared with 23% of the *CDKN2A*^{wt} cases and 35% of the sporadic cases. Causes of deaths among the melanoma cases of each identified *CDKN2A*^{mut} and *CDKN2A*^{wt} family are shown in Supplementary Table 1 and Supplementary Table 2 (available online). Of the 31 *CDKN2A*^{mut} families, 18 (58%) had deaths from melanoma, with one to three melanoma deaths per family and 12 (39%) families had deaths from nonmelanoma cancers, with one to three nonmelanoma cancer deaths per family. Of the 238 *CDKN2A*^{wt} families, 52 (22%) had deaths from melanoma, with one to two melanoma deaths per family, and 25 (11%) families had deaths from nonmelanoma cancers, all with one nonmelanoma cancer death per family. In conclusion, there were no individual families that accounted for any substantial portion of the mortalities. The family with the p.Pro48Leu mutation in *CDKN2A* had deaths from both melanoma and nonmelanoma cancer while the family with the p.delAla60_Gly67 mutation (n = 2) had no deaths from any cause among the melanoma cases. Causes of death subdivided depending on whether cases had been diagnosed with multiple primary melanomas or belonged to families with few (≤3) or many (≥4) members affected with melanoma are shown in Supplementary Table 3 and Supplementary Table 4, respectively (available online). In all subgroups, there were higher frequencies of death from melanoma and nonmelanoma cancer in *CDKN2A*^{mut} cases compared with *CDKN2A*^{wt} cases.

Survival Among *CDKN2A*^{mut}, *CDKN2A*^{wt}, and Sporadic Melanoma Cases

In the survival analyses, only cases with a diagnosis of invasive melanoma (for *CDKN2A*^{mut}, *CDKN2A*^{wt}, and sporadic melanoma cases: n = 96, n = 377, and n = 1.042, respectively) were included. In Figure 2, Kaplan-Meier plots are shown, demonstrating that in *CDKN2A*^{mut} familial cases compared with *CDKN2A*^{wt} cases statistically significantly worse survival was seen from all causes ($P = .03$), from melanoma ($P = .03$) and from nonmelanoma cancer ($P < .001$). In an unadjusted Cox proportional hazards model, age of 50 years or older was found to be statistically significantly associated with increased death rates from all causes, from melanoma and from nonmelanoma cancers (Table 3). Male sex was statistically significantly associated with higher death rates from all causes and from melanoma but not from nonmelanoma cancers. Higher T classification of the primary melanoma was associated with increased death rates from all causes and from melanoma and, interestingly, also with increased death rates from nonmelanoma cancer. In an unadjusted hazards model, *CDKN2A*^{mut} familial melanoma cases had, compared with *CDKN2A*^{wt} cases, worse survival from all causes (hazard ratio [HR] = 1.44, 95% confidence interval [CI] = 1.02 to 2.04), from melanoma (HR = 1.69, 95% CI = 1.04 to 2.75),

Table 3. Effect of age, sex, and tumor stage on survival in familial cases with invasive melanoma by univariate Cox proportional hazards regression model

	Survival from all causes		Survival from melanoma		Survival from nonmelanoma cancer	
Covariates	HR (95% CI)	P*	HR (95% CI)	P*	HR (95% CI)	P*
Age						
≥50 vs <50 y	3.86 (2.75 to 5.41)	<.001	1.68 (1.08 to 2.64)	.02	4.86 (2.24 to 10.60)	<.001
Sex						
Male vs female	1.64 (1.20 to 2.23)	.002	1.80 (1.14 to 2.83)	.01	1.43 (0.72 to 2.84)	.31
T classification†						
T2 vs T1	1.95 (1.29 to 2.94)	.01	2.17 (1.07 to 4.40)	.03	2.54 (1.16 to 5.58)	.02
T3 vs T1	4.18 (2.52 to 6.93)	.03	8.03 (3.94 to 16.35)	<.001	0.85 (0.11 to 6.49)	.33
T4 vs T1	7.80 (4.95 to 12.29)	<.001	18.30 (9.77 to 34.27)	<.001	4.79 (1.54 to 14.90)	.03
T2-T4 vs T1	3.08 (2.20 to 4.32)	<.001	5.45 (3.15 to 9.43)	<.001	2.51 (1.54 to 5.18)	.01

*All hazard ratio *P* values are two-sided. CI = confidence interval; HR = hazard ratio.
†T classification of first invasive melanoma tumor according to the 2009 American Joint Committee on Cancer melanoma staging and classification system.

and from nonmelanoma cancers (HR = 3.50, 95% CI = 1.76 to 6.94) (Table 4). After adjusting for age (age ≥ 50 vs age < 50 years), sex (male vs female), and T classification (T2-T4 vs T1), *CDKN2A*^{mut} familial melanoma cases had, compared with *CDKN2A*^{wt} cases, worse survival from all causes (HR = 2.59, 95% CI = 1.76 to 3.78), from melanoma (HR = 2.50, 95% CI = 1.49 to 4.21), and from nonmelanoma cancers (HR = 7.77, 95% CI = 3.65 to 16.51). The increase in hazard ratios after adjustments was mainly a result of the cohort of *CDKN2A*^{wt} cases being older and having more men, both factors related to a worse prognosis. Also, *CDKN2A*^{mut} cases had statistically significantly worse survival outcomes when compared with the matched sporadic cases (HR = 1.46, 95% CI = 1.01 to 2.09) while no statistically significant differences were found between *CDKN2A*^{wt} and sporadic cases (HR = 0.82, 95% CI = 0.66 to 1.01).

To assess whether death rates from melanoma were affected by multiple primary melanomas, an analysis was performed wherein all cases with multiple invasive primary melanomas were excluded. Also in this subgroup analysis, the *CDKN2A*^{mut} single melanoma cases had statistically significantly worse survival from all causes (HR = 3.92, 95% CI = 2.51 to 6.10), from nonmelanoma cancers (HR = 15.93, 95% CI = 6.50 to 39.04), and from melanoma (HR = 3.46, 95% CI = 1.92 to 6.24) compared with *CDKN2A*^{wt} single melanoma cases, both in the adjusted and in the unadjusted hazards models (Table 4). Similarly, to assess if melanoma-specific death rates were affected by diagnoses of other cancers (eg, deaths from other cancers misclassified as deaths from melanomas), an analysis was done where all cases with a diagnosis of nonskin cancers were excluded. Also in this subgroup, in the adjusted model, there was statistically significantly worse overall (HR = 2.23, 95% CI = 1.30 to 3.87) and melanoma-specific survival (HR = 2.33, 95% CI = 1.25 to 4.33). Furthermore, an analysis was performed wherein all cases diagnosed with either lung or pancreatic cancer were excluded. Interestingly, also with the pancreatic and lung cancer cases excluded, survival from nonmelanoma cancers was statistically significantly worse among the *CDKN2A*^{mut} cases (HR = 4.51, 95% CI = 1.11 to 18.30), indicating that other cancer types also contribute to the worse survival rates seen among the mutated cases.

Discussion

In this study, we show that familial melanoma cases with germline mutations in *CDKN2A* have younger age at onset, have increased numbers of melanoma cases per family, and are more prone to developing multiple melanomas and other cancers, compared with familial cases lacking *CDKN2A* mutations. No differences were seen between melanomas in *CDKN2A* mutation carriers compared with noncarriers with respect to melanoma-specific tumor features such as body site, invasiveness, or T classification of the primary tumor. However, *CDKN2A* mutation carriers had statistically significantly worse survival from all causes, from melanoma, and from nonmelanoma cancers, independent of sex, age, and T classification. Because Swedish *CDKN2A* mutation carriers previously have been shown to have an increased risk of developing nonmelanoma tumors (1,8), worse survival from other cancers was not an unexpected finding. Also, pancreatic and lung cancers, which are particularly common in carriers, are cancers with a poor prognosis. The finding of a statistically significantly worse melanoma-specific survival in mutation carriers was a novel and somewhat unexpected finding, albeit this result was unambiguous and independent of the covariates analyzed. We considered the possibility that the increased predisposition for multiple primary melanomas and other cancers in *CDKN2A* mutation carriers affected the melanoma-specific survival. Therefore, we performed analyses in which cases with multiple primary melanomas and nonskin cancers were excluded; however, also in these subgroups, melanoma-specific prognosis was statistically significantly worse in the *CDKN2A*^{mut} familial melanoma cases.

Our results indicate that the presence of a *CDKN2A* germline mutation is associated with a more aggressive phenotype of cutaneous melanoma, but the mechanism behind this is unclear. In a recent study, it was demonstrated that primary melanomas from *CDKN2A* mutation carriers do not exhibit a distinct gene expression signature compared with sporadic melanomas and, although *BRAF* mutations and *PTEN* losses were more frequent in melanomas from *CDKN2A* mutation carriers, these differences were not statistically significant after adjustments for tumor thickness and age (14). Still, somatic losses of *CDKN2A* have been associated with worse outcomes in melanoma (27–29) and also in various other cancers (gliomas, sarcomas, certain leukemias and lymphomas, lung, oral, gastroesophageal, renal cell, pancreatic, breast, bladder, hepatocellular cancers) (30–42).

Melanoma penetrance in *CDKN2A* mutation carriers has been shown to be associated with sun exposure patterns (2). Additionally, we have previously shown that *CDKN2A* mutation carriers have an increased incidence of tobacco-associated cancers (1). Hence, *CDKN2A* mutation carriers appear to be more

Table 4. Cox proportional hazards regression models for effect of *CDKN2A* mutation status on survival in familial and sporadic cases with invasive melanoma

Cohorts	Unadjusted survival from:						Adjusted* survival from:					
	All causes		Melanoma		Nonmelanoma cancer		All causes		Melanoma		Nonmelanoma cancer	
	HR (95% CI)	P†	HR (95% CI)	P†	HR (95% CI)	P†	HR (95% CI)	P†	HR (95% CI)	P†	HR (95% CI)	P†
$CDKN2A^{mut}$ (n = 96) vs $CDKN2A^{wt}$ (n = 377)	1.44 (1.02 to 2.04)	.04	1.69 (1.04 to 2.75)	.03	3.50 (1.76 to 6.94)	<.001	2.59 (1.76 to 3.78)	<.001	2.50 (1.49 to 4.21)	.001	7.77 (3.65 to 16.51)	<.001
$CDKN2A^{mut}$ (n = 65) vs $CDKN2A^{wt}$ (n = 343) w/ single invasive melanoma	2.07 (1.39 to 3.07)	<.001	1.93 (1.20 to 3.39)	.04	5.42 (2.57 to 11.44)	<.001	3.92 (2.51 to 6.10)	<.001	3.46 (1.92 to 6.24)	<.001	15.93 (6.50 to 39.04)	<.001
$CDKN2A^{mut}$ (n = 62) vs $CDKN2A^{wt}$ (n = 302) w/o diagnosis of nonskin cancer	1.23 (0.77 to 1.97)	.38	1.61 (0.93 to 2.79)	.09	—‡	—‡	2.23 (1.30 to 3.87)	.001	2.33 (1.25 to 4.33)	.001	—‡	—§
$CDKN2A^{mut}$ (n = 81) vs $CDKN2A^{wt}$ (n = 372) w/o diagnosis of pancreas/lung cancer	1.26 (0.85 to 1.87)	.25	1.88 (1.15 to 3.08)	.01	1.36 (0.45 to 4.14)	.59	2.67 (1.68 to 4.25)	<.001	3.36 (1.95 to 5.79)	<.001	4.51 (1.11 to 18.30)	.03
$CDKN2A^{mut}$ (n = 96) vs sporadic melanoma cases (n = 288)	1.46 (1.01 to 2.09)	.04	1.86 (1.11 to 3.10)	.02	2.30 (1.14 to 4.63)	.02	—§	—§	—§	—§	—§	—§
$CDKN2A^{wt}$ (n = 377) vs sporadic melanoma cases (n = 754)	0.82 (0.66 to 1.01)	.07	0.91 (0.66 to 1.26)	.57	0.72 (0.42 to 1.25)	.25	—§	—§	—§	—§	—§	—§

*Adjusted for age, sex, and T classification of the primary melanoma tumor. CI = confidence interval; $CDKN2A^{mut}$ = familial melanoma case with germline *CDKN2A* gene mutation; $CDKN2A^{wt}$ = familial melanoma case with no *CDKN2A* mutations (wild-type); HR = hazard ratio.
†All hazards ratio *P* values are two-sided.
‡Survival from nonmelanoma cancer not calculable because all cases diagnosed with nonskin cancer were excluded in this analysis.
§Adjustments for age, sex, and T classification not applicable because the sporadic melanoma cases were matched on the basis of these covariates.

sensitive to carcinogenic/mutagenic exposures. Interestingly, melanomas have been shown to be the cancer type that has the highest mutational load, which is accounted for largely by UV signature mutations (43). It is possible that germline or somatic mutations in the *CDKN2A* gene that lead to a disruption of proper cell cycle inhibition functions of the p16/p14ARF proteins permit cells with additional acquired mutations to progress through the cell cycle, causing increased accumulation of carcinogen-induced mutation in tumor cells, contributing to greater tumor aggressiveness. In this aspect, it would be of interest to compare the mutational load in tumors from germline *CDKN2A* mutation carriers and noncarriers, respectively, in particular mutations with carcinogen signatures.

This study shows that Swedish *CDKN2A* mutation carriers not only need thorough surveillance to prevent and detect melanomas at earlier stages, carriers may also benefit from more intense follow-up for melanoma recurrences. Earlier detection of stage III to IV melanoma will hopefully lead to better survival in this group, especially considering the landscape of effective melanoma regimens emerging. Activating *BRAF* mutations are at least as common in melanomas from *CDKN2A* mutation carriers as in sporadic melanomas (14,15), and such tumors may therefore be candidates for BRAF inhibitor–based therapies. Among *BRAF* mutation–positive melanoma cases receiving BRAF inhibitor therapy, somatic loss of *CDKN2A* has been associated with worse outcomes (44), but this might reflect the worse prognosis associated with *CDKN2A* loss rather than an association with worse response to BRAF inhibitors. Also, preclinical studies indicate that *CDKN2A* mutations may predict sensitivity in melanoma patients to CDK4/6 inhibitors that are emerging as promising novel kind of therapeutic agents in various cancers (45,46).

The main limitation of this study is the size of the $CDKN2A^{mut}$ melanoma cohort. This has to be considered in light of the fact that germline mutations in the *CDKN2A* gene are very rare in the normal population (<0.1%) (11). In this study, we included all *CDKN2A*-mutated melanoma families that have been identified in a national program in Sweden (current population = 10 million) aimed at detecting melanoma families, started in 1987. In spite of the relatively low number of mutation-carrying melanoma cases, the differences in survival rates between mutation carriers and noncarriers were so distinctive that statistically significant results were obtained. Also, this study reflects the situation of Swedish melanoma families where the main *CDKN2A* mutation found is the p.Arg112dup founder mutation and generalizability is uncertain until survival studies have been performed in other groups of *CDKN2A* mutation carriers. The strengths of this study are that all families have, independent of mutation status, been included in the same follow-up program and all data on family members (sex, dates of birth, death, and emigration), cancer diagnoses (dates, types, stages, body sites) were obtained from national registers with nearly full coverage that go back more than 50 years in time. Also, matched sporadic melanoma cases were included in the study, and this group had survival outcomes similar to the $CDKN2A^{wt}$ familial cases, further corroborating the finding of poor outcomes in the $CDKN2A^{mut}$ cases.

To summarize, we present the first study that estimates survival in familial melanoma cases depending on *CDKN2A* mutation status. The finding of statistically significantly worse survival in Swedish *CDKN2A* mutation carriers needs to be confirmed in other familial melanoma cohorts but nonetheless raises important questions regarding the possible underlying biological processes. Also, this finding indicates that *CDKN2A*

mutation carriers not only need thorough surveillances aimed at prevention and earlier detection of primary tumors but also may benefit from more intense follow-up for melanoma recurrences.

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