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Article

Impact of Transcript (p16/p14ARF) Alteration on Cancer Risk in *CDKN2A* Germline Pathogenic Variant Carriers

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Abstract

Background: Few studies have evaluated the relationship between *CDKN2A* germline pathogenic variants (GPV), transcript (p16/p14ARF) alteration, and cancer risk. **Methods:** Standardized incidence ratios (SIRs) comparing cancer risk with the general population were calculated for 385 *CDKN2A* GPV carriers from 2 large cohorts (259 United States and 126 Swedish individuals) using Poisson regression; statistical significance was defined as *P* less than .002 (Bonferroni correction). Cumulative incidence is reported for melanoma and nonmelanoma cancer. **Results:** Incidence was increased for melanoma (SIR = 159.8, 95% confidence interval [CI] = 132.1 to 193.2), pancreatic cancer (SIR = 24.1, 95% CI = 14.7 to 39.4), head and neck squamous cell carcinoma (SIR = 16.2, 95% CI = 9.5 to 27.6), and lung cancer (SIR = 5.6, 95% CI = 3.4 to 9.1) in GPV carriers. Similar associations were observed with p16 alteration. Combined p16 and p14ARF alteration was associated with increased incidence of esophageal cancer (SIR = 16.7, 95% CI = 5.7 to 48.9) and malignant peripheral nerve sheath tumor (SIR = 113.0, 95% CI = 16.4 to 780.9), although cancer events were limited ($n < 5$ for each malignancy). Cumulative incidence at age 70 years for melanoma and nonmelanoma cancer was 68.3% (95% CI = 68.0% to 68.6%) and 35.2% (95% CI = 34.9% to 35.6%), respectively. A total 89% of smoking-related cancers (lung, head and neck squamous cell carcinoma, pancreatic, esophageal) occurred in ever smokers. **Conclusion:** These findings highlight the impact of p16 and p14ARF alteration on cancer risk. Smoking was an important risk factor for smoking-related cancers in our study.

Genetic alterations of *cyclin-dependent kinase inhibitor 2A* (*CDKN2A*), the major high-risk melanoma susceptibility gene, can alter the function of tumor suppressor proteins p16 and/or p14ARF (1-3). Functional impairment of these proteins increases susceptibility to melanoma by causing downstream inhibition of Rb (affected by p16 alteration) and p53 (affected by p14ARF alteration), important regulators of cell division (3).

Multiple nonmelanoma cancer types have been reported in *CDKN2A* germline pathogenic variant (GPV) carriers, including pancreatic, breast, brain (astrocytoma), head and neck, respiratory tract, gynecological, digestive, and soft tissue (4-13). However, results on cancer risk in this population have been inconsistent except for pancreatic cancer (4-13). Multiple factors may explain the lack of reproducible findings between studies, including small sample sizes; variable follow-up; differences in cancer susceptibility between study populations based on, for example, polygenic risk and environmental exposures; and the inclusion of self-reported cancer diagnoses in some analyses. Most previous analyses also did not adjust for family relatedness or alterations of p16 and/or p14ARF, which could further affect associations of cancer risk. Intensive skin surveillance (and perhaps greater ultraviolet protection) has also reduced melanoma incidence and thickness in people with *CDKN2A* GPVs, which may improve survival and allow for the development of nonmelanoma cancers later in life (14,15).

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In this study, we analyze cancer risk for large United States and Swedish cohorts of *CDKN2A* GPV carriers. We use long-term follow-up data to characterize the spectrum of cancer risk in these 2 cohorts and determine whether alterations of specific transcripts (p16 and p14ARF) are associated with cancer incidence. We report cumulative incidence to help guide screening recommendations for nonmelanoma cancer types.

Methods

Study Population

The US cohort of GPV carriers was identified from a natural history study of families with at least 2 individuals with melanoma followed at the National Institutes of Health (NCT00040352) (15). GPV carriers were considered at risk for cancer starting at birth and were followed up until death or date of last contact, up to December 31, 2020. After a cancer diagnosis, individuals were followed up for the occurrence of additional malignancies at separate primary sites until death or date of last contact. All included cancer diagnoses were confirmed by pathology review and/or with medical records (eg, pathology reports). Death information was confirmed by medical records, death certificates, or a search of the National Death Index in December 2021. Smoking information was collected through questionnaires mailed to study participants or from medical records (eg, physician notes). Individuals were considered ever smokers if they smoked at least 100 cigarettes in their lifetime. Additional information about this US cohort has been described in previous publications (2,15). Written informed consent was obtained from all participants in NCT00040352 (approved by National Institutes of Health institutional review board).

The Swedish cohort included all individuals with *CDKN2A* GPVs identified in Swedish medical clinics from January 1, 1987, through December 31, 2011. Individuals were referred for *CDKN2A* testing in Sweden if at least 1 first-, second-, or third-degree relative had melanoma that was verified by pathology and/or medical records. Follow-up started at birth and ended at death or the last date of cancer data collection, December 31, 2011. National 10-digit personal identity numbers for GPV carriers were linked with the Swedish Cancer Registry to identify cancer diagnoses and the Cause of Death Registry to obtain date and cause of death. After a cancer diagnosis, individuals were followed up for the occurrence of additional malignancies at separate primary sites until death or December 31, 2011. Smoking information was obtained from questionnaires administered to Swedish GPV carriers. Similarly to the US cohort, ever smokers were defined as having smoked 100 or more cigarettes during their lifetime (9). Data collection for the Swedish cohort was approved by the Swedish ethical review authority.

GPV carriers in both cohorts were identified through gene evaluation (eg, sequencing, genetic testing) or were obligate carriers (based on pedigree). Alterations of p16 and p14ARF were determined based on variant position and subsequent protein change as described in prior publications (Supplementary Figure 1, available online) (16,17). Three families with GPVs of *CDKN2A* and a second cancer predisposition gene were excluded from the analyses: 2 Swedish families with confirmed pathogenic *BRCA1* variants and 1 US family with a confirmed pathogenic *ATM* variant.

Statistical Analysis

The ratio of the observed to expected number of cancers, the standardized incidence ratio (SIR), was used to compare cancer risk in GPV carriers with the general population. Person-time for each cohort member was computed from birth to cohort exit, defined as the earliest of the following events: death, end of follow-up (December 31, 2020, for US GPV carriers; December 31, 2011, for Swedish GPV carriers) or known date of last contact. For US GPV carriers, year, sex, and age-specific (5-year age groups) cancer incidence rates from the Surveillance, Epidemiology, and End Results 9 and 18 cancer registries (18) were used to determine expected cancer counts for years 1975-1999 and 2000-2016, respectively. Rates were restricted to Non-Hispanic White individuals to match US GPV carriers. Surveillance, Epidemiology, and End Results 9 1975 rates were used to estimate the expected number of cancers diagnosed before 1975. Expected counts for each cancer type were calculated for Swedish GPV carriers using year, sex, and age-specific (5-year age groups) cancer incidence rates from the Swedish cancer registry. Sweden has a majority White population, and therefore we assumed that rates were representative of White individuals. Variance estimates and 95% confidence intervals (CIs) for the SIRs were obtained assuming that the observed number of events follows a Poisson distribution. To accommodate correlations from individuals in the same family, we corrected the Poisson variance by multiplying it by the overdispersion factor and then computed 95% confidence intervals and *P* values based on the corrected variance. SIR calculations were restricted to the first diagnosis of each cancer type, and first primary cancer rates were used to estimate expected counts.

We estimated the cumulative incidence function, which is the probability of developing a particular outcome in the presence of competing mortality, for cancer overall and specific cancer types nonparametrically using the Aalen-Johansen estimator as implemented in proc life-test in SAS 9.4 (19). Follow-up started at birth and ended at age at cancer diagnosis, death, or end of follow-up.

All *P* values were 2-sided, and we applied a Bonferroni correction to account for multiple comparisons ($n = 22$ cancer types), which resulted in a cutoff for statistical significance of *P* less than .002. To assess the potential effect of ascertainment bias on cancer risk estimates, we repeated the SIR computations in a sensitivity analysis in which follow-up time started at the time of family ascertainment for US and Swedish GPV carriers.

Results

Demographic, clinical, and genomic characteristics are reported for 385 individuals in Table 1, which includes 259 (67%) US and 126 (33%) Swedish GPV carriers, respectively. The study population was predominantly female (54% female, 46% male) and comprised of White individuals (100% in US cohort; information on race was not available for Swedish cohort). The median age at first cancer diagnosis was 37 years (range = 9-83 years). Fifty percent of the study population developed at least 1 invasive cancer, and 12% developed 2 or more unique cancer types during follow-up. *CDKN2A* variants altering both p16 and p14ARF were detected in most individuals (62%), followed by variants altering p16 only (31%) or p14ARF only (6%). Ninety-four percent of Swedish GPV carriers had alteration of both transcripts due chiefly to the Swedish founder variant (p.Arg112dup, Supplementary Figure 1, available online).

Table 1. Characteristics of individuals with *CDKN2A* germline pathogenic variants

Clinical characteristics	All variant carriers, No. (%)	US variant carriers, No. (%)	Swedish variant carriers, No. (%)
Total	385 (100)	259 (100)	126 (100)
Sex			
Female	208 (54)	131 (51)	77 (61)
Male	177 (46)	128 (49)	49 (39)
Race			
White	—[a]	259 (100)	—[a]
Age at first cancer diagnosis, y			
9-17	13 (5)	12 (7)	1 (1)
18-29	63 (26)	48 (28)	15 (21)
30-49	105 (43)	64 (37)	41 (57)
50-64	44 (18)	32 (19)	12 (17)
65-83	18 (7)	15 (9)	3 (4)
Median age (range)	37 (9-83)	37 (9-83)	37 (16-75)
Birth year			
1887-1949	143 (37)	101 (39)	42 (33)
1950-1959	79 (21)	58 (22)	21 (17)
1960-1969	62 (16)	37 (14)	25 (20)
1970-1996	101 (26)	63 (24)	38 (30)
Calendar period of first cancer diagnosis			
All calendar periods	243 (100)	171 (100)	72 (100)
1944-1974	51 (21)	43 (25)	8 (11)
1975-1989	83 (34)	60 (35)	23 (32)
1990-2003	67 (28)	40 (23)	27 (38)
2004-2020	42 (17)	28 (16)	14 (19)
Unique invasive cancer types, no.			
None	144 (37)	90 (35)	54 (43)
1	194 (50)	142 (55)	52 (41)
2-3	47 (12)	27 (10)	20 (16)
Transcripts altered by variant			
p16 only	121 (31)	113 (44)	8 (6)
p16 and p14ARF	240 (62)	122 (47)	118 (94)
p14ARF only	24 (6)	24 (9)	0

[a]Information on race was not available for Swedish *CDKN2A* carriers.

There were 200 GPV carriers who developed invasive melanoma during 15 666.9 person-years of follow-up, corresponding to a 159.8-fold (95% CI = 132.1- to 193.2-fold) increased incidence compared with the general population. Melanoma incidence remained increased when follow-up time was restricted to after the family ascertainment date (SIR = 81.8, 95% CI = 58.3 to 114.8) (Table 2). Twenty-one percent (n = 79) of the study population developed at least 1 nonmelanoma cancer. The most common nonmelanoma cancers were lung (n = 23 GPV carriers), pancreas (n = 19 GPV carriers), and head and neck squamous cell carcinoma (HNSCC; n = 14 GPV carriers), and incidence for each of these cancers was elevated compared with the general population after Bonferroni correction (SIRs = 5.6 [95% CI = 3.4 to 9.1] for lung, 24.1 [95% CI = 14.7 to 39.4] for pancreas, 16.2 [95% CI = 9.5 to 27.6] for HNSCC; $P < .001$ for all associations; similar estimates were observed for each cancer type when follow-up time was restricted to after the family ascertainment date). To assess the impact of using 1975 cancer incidence rates to estimate expected cancer counts for pre-1975 years, we estimated SIRs restricted to events and person-years accumulated after 1975 and obtained similar results (Supplementary Table 1, available online). Smoking information was available for 63% (25 of 40 GPV carriers) of US and 63% (12 of 19 GPV carriers) of Swedish GPV carriers who developed a smoking-related malignancy (lung, HNSCC, pancreatic, esophageal). Overall, 89% of smoking-related cancers occurred in ever smokers, including 100% of lung cancers (14 of 14 GPV carriers; cigarette use unknown for 9 GPV carriers), 75% of HNSCCs (9 of 12 GPV carriers; cigarette use unknown for 2 GPV carriers), and 90% of pancreatic cancers (9 of 10 GPV carriers; cigarette use unknown for 9 GPV carriers).

Individuals with variants altering p16 only (p14ARF unaltered) had statistically significant elevations ($P < .001$) in risk for invasive melanoma (SIR = 157.5, 95% CI = 118.7 to 209.0), pancreatic cancer (SIR = 25.0, 95% CI = 10.4 to 60.1), lung cancer (SIR = 7.2, 95% CI = 3.5 to 14.5), and HNSCC (SIR = 12.6, 95% CI = 4.1 to 38.8) (Table 3). Incidence for these cancers was also elevated ($P < .001$) in people with variants altering both p16 and p14ARF, and SIRs did not differ between the 2 groups (Supplementary Table 2, available online). Furthermore, although based on few events, individuals with variants altering both p16 and p14ARF had a Bonferroni-corrected statistically significant increased incidence of esophageal cancer (n = 3 GPV carriers; SIR =16.7, 95% CI = 5.7 to 48.9, $P < .001$), malignant peripheral nerve sheath tumor (n = 2 GPV carriers; SIR = 113.0, 95% CI = 16.4 to 780.9, $P < .001$), and adrenal gland cancer (n = 1 GPV carrier; SIR = 108.2, 95% CI = 14.9 to 786.0, $P < .001$) (Table 3). There also was an increased incidence for leukemia (n = 3 GPV carriers; SIR = 4.2, 95% CI = 1.5 to 12.3, $P = .008$; 1 case of acute lymphoblastic leukemia and 2 cases of leukemia, not otherwise specified) in people with variants altering both transcripts, but this association was not statistically significant after Bonferroni correction. In contrast, people with variants altering p14ARF only (p16 unaltered) were at increased risk for melanoma but not for any nonmelanoma cancers.

Both US and Swedish GPV carriers had a statistically significant increased ($P < .001$) incidence for invasive melanoma, pancreatic cancer, lung cancer, and HNSCC (Table 4). Compared with the Swedish cohort, elevation in incidence for nonmelanoma cancers was lower for US GPV carriers (ratio of SIR-US to SIR-Sweden = 0.6, 95% CI = 0.3 to 0.97) (Supplementary Table 3, available online).

By ages 50 and 70 years, cumulative incidence of invasive melanoma was 50.9% (95% CI = 50.6% to 51.1%) and 68.3% (95% CI = 68.0% to 68.6%), respectively (Figure 1). For all nonmelanoma cancers, cumulative incidence was 10.6% (95% CI = 10.4% to 10.7%) at age 50 years and 35.2% (95% CI = 34.9% to 35.6%) at age 70 years. Cumulative incidence for nonmelanoma cancers started to increase substantially after age 50 years and reached 5.9% (95% CI = 5.7% to 6.0%) for HNSCC, 7.8% (95% CI = 7.6% to 7.9%) for pancreatic cancer, and 10.1% (95% CI = 9.8% to 10.3%) for lung cancer by age 70 years. The rapid rise in nonmelanoma cancer cumulative incidence after age 50 years was observed for both US (Supplementary Figure 2, available online) and Swedish (Supplementary Figure 3, available online) GPV carriers. Cumulative incidence estimates for nonmelanoma cancer did not differ between individuals with variants altering p16 only and individuals with variants altering both p16 and p14ARF (Supplementary Figure 4, available online).

Detailed information about *CDKN2A* variants is available in Supplementary Table 4 (available online).

Table 2. SIRs for individuals with *CDKN2A* germline pathogenic variants

Cancer site	Cancers diagnosed before or after family ascertainment date				Cancers diagnosed after family ascertainment date			
	Person-years	No. of events[a]	SIR (95% CI)[b]	P[c]	Person-years	No. of events[a]	SIR (95% CI)[b]	P[c]
All cancer sites[d]	15 400.1	243	13.6 (11.5 to 16.0)	<.001	3734.1	88	8.9 (6.8 to 11.5)	<.001
All cancer sites except invasive melanoma[e]	18 710.2	79	2.4 (1.8 to 3.2)	<.001	5875.7	54	2.4 (1.7 to 3.4)	<.001
Invasive melanoma	15 666.9	200	159.8 (132.1 to 193.2)	<.001	3889.9	59	81.8 (58.3 to 114.8)	<.001
Pancreas	19 255.7	19	24.1 (14.7 to 39.4)	<.001	6275.9	14	24.0 (13.3 to 43.1)	<.001
Esophagus	19 265.1	3	9.4 (3.1 to 28.8)	.001	6283.1	3	12.5 (4.1 to 38.1)	<.001
Colorectal	19 211.6	5	1.4 (0.6 to 3.2)	.47	6231.9	4	1.6 (0.6 to 3.9)	.35
Stomach	19 267.7	1	1.7 (0.2 to 11.3)	.60	6285.7	1	2.6 (0.4 to 17.5)	.34
Lung and bronchus	19 226.6	23	5.6 (3.4 to 9.1)	<.001	6262.0	17	5.8 (3.2 to 10.6)	<.001
Head and neck squamous cell carcinoma	19 116.5	14	16.2 (9.5 to 27.6)	<.001	6180.4	7	12.3 (6.0 to 25.2)	<.001
Female breast	10 334.4	9	1.3 (0.8 to 2.3)	.34	3453.7	5	1.1 (0.5 to 2.2)	.86
Ovary	10 447.9	2	2.3 (0.6 to 8.1)	.21	3539.8	2	3.9 (1.1 to 14.4)	.04
Prostate	8756.7	6	1.4 (0.6 to 3.6)	.44	2683.2	5	1.4 (0.6 to 3.5)	.42
Sarcoma	19 178.0	3	6.4 (1.5 to 26.9)	.01	6232.6	1	4.1 (0.6 to 28.5)	.16
Malignant peripheral nerve sheath tumor	19 219.5	2[f]	72.8 (10.4 to 509.3)	<.001	6260.0	1[g]	85.0 (12.2 to 590.9)	<.001
Glioblastoma	19 263.3	2	4.8 (1.3 to 18.1)	.02	6281.2	2	8.2 (2.2 to 31.0)	.002
Blood	19 229.2	6	1.6 (0.8 to 3.3)	.20	6267.1	4	1.7 (0.7 to 4.4)	.26
Leukemia	19 249.8	3	2.4 (0.8 to 7.3)	.12	6267.8	3	4.2 (1.4 to 12.8)	.01
Lymphoma	19 247.3	2	1.0 (0.2 to 3.8)	.95	6285.2	0	—	
Myeloma	19 267.9	1	2.4 (0.4 to 15.5)	.36	6285.8	1	3.2 (0.5 to 21.2)	.24
Renal cell carcinoma	19 266.9	2	2.3 (0.6 to 9.3)	.24	6284.9	2	3.1 (0.8 to 12.7)	.11
Adrenal gland cancer	19 267.6	1	67.0 (9.3 to 484.2)	<.001	6285.5	1	113.3 (15.6 to 824.6)	<.001
Thyroid	19 247.4	1	1.0 (0.1 to 7.6)	.97	6279.0	0	—	

[a]Number of specific cancers diagnosed during the person-years of follow-up. CI = confidence interval; SIR = standardized incidence ratio.

[b]SIRs were calculated for United States (n = 259) and Swedish (n = 126) individuals with *CDKN2A* germline pathogenic variants. For the combined SIRs, the observed number of events for US and Swedish cohort members were summed and divided by the US and Swedish person-years multiplied by the respective rates. To accommodate correlations from individuals in the same family, we corrected the Poisson variance by multiplying it by the overdispersion factor and then computed 95% confidence intervals and *P* values based on the corrected variance. Analyses were restricted to the first diagnosis of each cancer type. No SIR was calculated if there were zero cancer events (symbol "—").

[c]Statistical significance was defined as *P* less than .002 based on a Bonferroni correction (alpha = .05/22 comparisons) to account for multiple comparisons.

[d]Analysis was restricted to first invasive cancer diagnoses, and therefore the total number of cases in this category does not equal the sum of first diagnoses for specific cancer types.

[e]Analysis was restricted to first invasive cancer diagnoses (excluding melanoma), and therefore the total number of cases in this category does not equal the sum of first diagnoses for specific cancer types (excluding melanoma).

[f]Genetic testing was negative for an *NF1* mutation or deletion in 1 individual and was not performed in the second individual.

[g]Genetic testing was negative for an *NF1* mutation or deletion in this individual.

Table 3. SIRs stratified by transcript alteration

Cancer site	p16 Altered/p14ARF unaltered				p16 Altered/p14ARF altered				p16 Unaltered/p14ARF altered[a]			
	Person-years	No. of events[b]	SIR (95% CI)[c]	P[d]	Person-years	No. of events[b]	SIR (95% CI)[c]	P[d]	Person-years	No. of events[b]	SIR (95% CI)[c]	P[d]
All cancer sites[e]	4865.5	84	12.1 (9.3 to 15.7)	<.001	9706.3	140	14.0 (11.3 to 17.3)	<.001	828.3	19	19.9 (11.1 to 35.5)	<.001
All cancer sites except invasive melanoma[f]	5833.7	30	2.6 (1.6 to 4.4)	<.001	11654.0	48	2.5 (1.8 to 3.5)	<.001	1222.6	1	0.4 (0.1 to 1.2)	.11
Invasive melanoma	4928.9	67	157.5 (118.7 to 209.0)	<.001	9909.6	114	148.4 (115.8 to 190.2)	<.001	828.3	19	327.4 (198.0 to 541.5)	<.001
Pancreas	6006.8	7	25.0 (10.4 to 60.1)	<.001	12023.6	12	26.6 (15.1 to 47.1)	<.001	1225.3	0	—	
Esophagus	6011.1	0	—		12028.8	3	16.7 (5.7 to 48.9)	<.001	1225.3	0	—	
Colorectal	5982.0	1	0.8 (0.1 to 5.7)	.82	12004.3	4	1.9 (0.8 to 4.6)	.18	1225.3	0	—	
Stomach	6011.1	0	—		12031.3	1	2.8 (0.4 to 18.1)	.27	1225.3	0	—	
Lung and bronchus	5999.6	13	7.2 (3.5 to 14.5)	<.001	12004.5	9	4.5 (2.2 to 9.3)	<.001	1222.6	1	3.4 (1.1 to 10.1)	.03
Head and neck squamous cell carcinoma	5959.2	4	12.6 (4.1 to 38.8)	<.001	11932.1	10	20.4 (11.5 to 36.4)	<.001	1225.3	0	—	
Female breast	3022.2	4	2.0 (1.2 to 3.4)	.01	6712.3	5	1.2 (0.5 to 2.6)	.73	600.0	0	—	
Ovary	3071.8	0	—		6776.1	2	3.4 (1.0 to 11.6)	.046	600.0	0	—	
Prostate	2904.7	3	1.9 (0.4 to 8.5)	.43	5226.7	3	1.3 (0.4 to 3.8)	.68	625.3	0	—	
Sarcoma	6011.1	0	—		11941.6	3	9.1 (2.2 to 37.9)	.002	1225.3	0	—	
Malignant peripheral nerve sheath tumor	6011.1	0	—		11983.1	2[g]	113.0 (16.4 to 780.9)	<.001	1225.3	0	—	
Glioblastoma	6010.8	1	8.1 (1.3 to 51.6)	.03	12027.2	1	3.7 (0.6 to 24.4)	.17	1225.3	0	—	
Blood	6011.1	1	0.8 (0.1 to 4.3)	.76	11992.8	5	2.3 (1.1 to 5.1)	.04	1225.3	0	—	
Leukemia	6011.1	0	—		12013.4	3	4.2 (1.5 to 12.3)	.008	1225.3	0	—	
Lymphoma	6011.1	0	—		12010.9	2	1.7 (0.4 to 6.3)	.46	1225.3	0	—	
Myeloma	6011.1	1	7.2 (1.3 to 40.2)	.03	12031.5	0	—		1225.3	0	—	
Renal cell carcinoma	6010.3	1	3.4 (0.5 to 25.3)	.23	12031.4	1	2.0 (0.3 to 14.0)	.50	1225.3	0	—	
Adrenal gland cancer	6011.1	0	—		12031.2	1	108.2 (14.9 to 786.0)	<.001	1225.3	0	—	
Thyroid	6011.1	0	—		12011.0	1	1.9 (0.3 to 14.5)	.52	1225.3	0	—	

[a]All individuals were from the United States cohort. CI = confidence interval; SIR = standardized incidence ratio.

[b]Number of specific cancers diagnosed during the person-years of follow-up.

[c]The impact of transcript alteration (p16 and/or p14ARF) on SIRs for specific cancers was evaluated for US (n = 259) and Swedish (n = 126) individuals with germline pathogenic variants in *CDKN2A*. For the combined SIRs, the observed number of events for US and Swedish cohort members were summed and divided by the US and Swedish person-years multiplied by the respective rates. To accommodate correlations from individuals in the same family, we corrected the Poisson variance by multiplying it by the overdispersion factor and then computed 95% confidence intervals and *P* values based on the corrected variance. Analyses were restricted to the first diagnosis of each cancer type. No SIR was calculated if there were zero cancer events (symbol "—").

[d]Statistical significance was defined as *P* less than .002 based on a Bonferroni correction (alpha = .05/22 comparisons) accounting for multiple comparisons.

[e]Analysis was restricted to first invasive cancer diagnoses, and therefore the total number of cases in this category does not equal the sum of first diagnoses for specific cancer types.

[f]Analysis was restricted to first invasive cancer diagnoses (excluding melanoma), and therefore the total number of cases in this category does not equal the sum of first diagnoses for specific cancer types (excluding melanoma).

[g]Genetic testing was negative for an *NF1* mutation or deletion in 1 individual and was not performed in the second individual.

Table 4. SIRs for US and Swedish cohorts

	United States				Sweden			
Cancer site	Person-years	No. of events[a]	SIR (95% CI)[b]	P[c]	Person-years	No. of events[a]	SIR (95% CI)[b]	P[c]
All cancer sites[d]	10 095.3	171	12.7 (10.7 to 15.1)	<.001	5304.9	72	16.1 (10.8 to 24.1)	<.001
All cancer sites except invasive melanoma[e]	12 379.5	53	2.1 (1.5 to 3.1)	<.001	6330.7	26	3.2 (2.3 to 4.4)	<.001
Invasive melanoma	10 276.4	139	160.3 (130.5 to 196.8)	<.001	5390.4	61	158.7 (105.4 to 238.8)	<.001
Pancreas	12 739.1	13	21.2 (11.4 to 39.2)	<.001	6516.6	6	34.1 (15.7 to 74.2)	<.001
Esophagus	12 747.2	1	4.1 (0.6 to 28.4)	.16	6517.9	2	27.5 (8.0 to 94.5)	<.001
Colorectal	12 696.3	3	1.1 (0.4 to 3.4)	.83	6515.2	2	2.0 (0.5 to 7.5)	.29
Stomach	12 747.2	0	—		6520.5	1	5.5 (1.0 to 31.5)	.05
Lung and bronchus	12 715.2	19	5.1 (3.0 to 8.8)	<.001	6511.5	4	9.7 (3.8 to 24.9)	<.001
Head and neck squamous cell carcinoma	12 692.0	7	10.5 (4.9 to 22.2)	<.001	6424.6	7	35.5 (17.0 to 74.3)	<.001
Female breast	6411.4	6	1.3 (0.7 to 2.5)	.44	3923.0	3	1.3 (0.5 to 3.6)	.58
Ovary	6496.4	0	—		3951.5	2	6.3 (2.2 to 17.8)	<.001
Prostate	6213.5	4	1.3 (0.4act to 4.4)	.66	2543.2	2	1.8 (0.5 to 6.3)	.37
Sarcoma	12 657.4	3	12.9 (3.2 to 52.1)	<.001	6520.6	0	—	
Malignant peripheral nerve sheath tumor	12 698.8	2[f]	120.0 (17.2 to 834.7)	<.001	6520.6	0	—	
Glioblastoma	12 747.0	1	3.9 (0.6 to 26.3)	.16	6516.3	1	6.1 (1.0 to 38.5)	.05
Blood	12 731.6	3	1.1 (0.4 to 3.0)	.92	6497.6	3	3.3 (1.3 to 8.7)	.01
Leukemia	12 733.7	1	1.1 (0.2 to 7.5)	.94	6516.1	2	6.6 (2.0 to 21.9)	.002
Lymphoma	12 745.2	1	0.6 (0.1 to 4.2)	.63	6502.1	1	2.1 (0.3 to 14.5)	.46
Myeloma	12 747.2	1	3.3 (0.5 to 21.0)	.20	6520.6	0	—	
Renal cell carcinoma	12 746.4	1	1.6 (0.2 to 11.3)	.65	6520.6	1	4.3 (0.6 to 30.3)	.14
Adrenal gland cancer	12 747.2	0	—		6520.3	1	198.3 (27.1 to 1450.0)	<.001
Thyroid	12 747.2	0	—		6500.1	1	6.2 (0.8 to 46.0)	.08

[a]Number of specific cancers diagnosed during the person-years of follow-up. CI = confidence interval; SIR = standardized incidence ratio.

[b]Observed cancer counts were divided by the expected number of cases for each cancer type to calculate SIRs for US (n = 259) and Swedish (n = 126) individuals with *CDKN2A* germline pathogenic variants. To accommodate correlations from individuals in the same family, we corrected the Poisson variance by multiplying it by the overdispersion factor and then computed 95% confidence intervals and *P* values based on the corrected variance. Analyses were restricted to the first diagnosis of each cancer type. No SIR was calculated if there were zero cancer events (symbol "—").

[c]Statistical significance was defined as *P* less than .002 based on a Bonferroni correction (alpha = .05/22 comparisons) accounting for multiple comparisons.

[d]Analysis was restricted to first invasive cancer diagnoses, and therefore the total number of cases in this category does not equal the sum of first diagnoses for specific cancer types.

[e]Analysis was restricted to first invasive cancer diagnoses (excluding melanoma), and therefore the total number of cases in this category does not equal the sum of first diagnoses for specific cancer types (excluding melanoma).

[f]Genetic testing was negative for an *NF1* mutation or deletion in 1 individual and was not performed in the second individual.

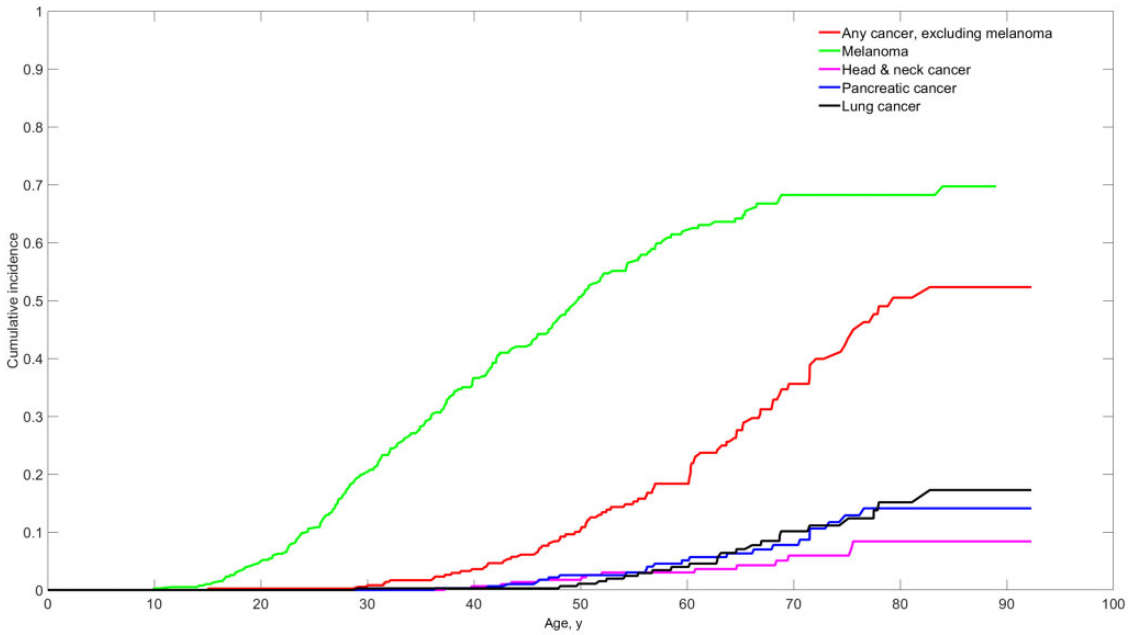

Figure 1. Cumulative incidence of melanoma and the most prevalent nonmelanoma cancer types in individuals with *CDKN2A* germline pathogenic variants. Cumulative incidence accounting for competing mortality was estimated nonparametrically for melanoma and nonmelanoma cancer types in US and Swedish *CDKN2A* germline pathogenic variant carriers using the Aalen-Johansen estimator. Follow-up started at birth and ended at incidence of the cancer of interest, death from other causes, or end of follow-up.

Discussion

Consistent with previous studies, GPV carriers had a higher incidence of invasive melanoma, pancreatic cancer, HNSCC, and lung cancer compared with the respective general populations (4-13). Although incidence was statistically significantly elevated for esophageal cancer, malignant peripheral nerve sheath tumor, and adrenal gland cancer and statistically nonsignificantly elevated for leukemia among individuals with GPV altering both p16 and p14ARF, further studies are needed to confirm these associations given the low number of events for each cancer type. Long-term follow-up data for our study cohorts and the possibility that rare cancers might be associated with specific *CDKN2A* variants that were absent from previous smaller studies could potentially explain these associations. The expanded spectrum of cancer risk might also be explained by the additive effect of disrupting both Rb (affected by p16 alteration) and p53 (affected by p14ARF alteration) signaling (3). The high proportion (94%) of individuals in Sweden with alteration of both p16 and p14ARF was due to a founder mutation, and 20 unique variants were identified in the US cohort, reflecting the genetic diversity of that population. Incidence of nonmelanoma cancers was not increased for individuals with variants altering p14ARF only, although this observation was based on only 19 individuals.

Both US and Swedish GPV carriers had an increased incidence of smoking-related cancers, which is consistent with previous studies (7-9). Eighty-nine percent of smoking-related cancers (pancreatic, lung, HNSCC, esophageal) in the study population occurred in ever smokers, suggesting that cigarette use likely affects susceptibility to these malignancies. Furthermore, although cancer incidence was generally similar between the US and Swedish cohorts, incidence for nonmelanoma cancers was 40% lower in the US cohort than the Swedish cohort, and this could reflect variation in genetic risk factors (eg, polygenic risk, dissimilar pathogenic variants; Supplementary Figure 1, available online) and/or environmental exposures between populations.

In this study, 4 GPV carriers (1.0% of study population) developed neural tumors (2 malignant peripheral nerve sheath tumors, 2 glioblastomas), consistent with *CDKN2A*-associated melanoma-astrocytoma syndrome (3). Previous studies have reported melanoma-astrocytoma syndrome in people with large deletions or splice site variants of *CDKN2A* that alter both p16 and p14ARF (20-25). In this study, 3 GPV carriers with neural tumors had variants altering both p16 and p14ARF, whereas the fourth individual (diagnosis of glioblastoma) had a variant altering p16 only.

Like previous estimates, cumulative incidence for melanoma at age 70 years was 68.3% (26). The study population was also susceptible to developing nonmelanoma cancers, particularly after age 50 years, with a cumulative incidence of 35.2% by age 70 years. For pancreatic cancer, cumulative incidence (7.8% at age 70 years) was similar to that observed in an Italian cohort of *CDKN2A* GPV carriers (cumulative incidence at age 75 years, 6.7%) but was lower than the cumulative incidence observed in a Dutch cohort (cumulative incidence at age 75 years, 19%) (11,13). The higher cumulative incidence for pancreatic cancer in Dutch GPV carriers could be related to environmental exposures such as smoking, which is more prevalent in the Netherlands than in the United States (27,28) or differences in distribution of *CDKN2A* GPVs. Additional studies in diverse populations of *CDKN2A* GPV carriers are needed to further refine cumulative incidence estimates and systematically evaluate the impact of smoking on cancer susceptibility.

Intensive skin surveillance is recommended for our study population because of their highly elevated risk for melanoma. Some experts also recommend radiologic surveillance for pancreatic cancer, which has been associated with the diagnosis of earlier-stage disease and improved survival for *CDKN2A* GPV carriers, although debate still exists on this matter, with the US Preventive

Services Task Force calling for additional studies (29-33). Further studies are also needed to determine whether lung cancer screening would improve outcomes in GPV carriers who had a 10.1% cumulative incidence for this cancer type by age 70 years.

Our study had several limitations that could affect the interpretation of results. All US GPV carriers were White individuals (race unknown for Swedish GPV carriers), and therefore estimates of cancer risk may not be applicable to Non-White individuals with GPV in *CDKN2A*. Variant carriers in the United States and Sweden were ascertained based on a family history of melanoma, which could have inflated estimates of melanoma incidence. However, melanoma incidence remained highly elevated when restricting follow-up time to after the family ascertainment date. In the United States and Sweden, population cancer data became available in 1975. We therefore estimated expected cancer counts for years 1944 to 1974 using 1975 incidence rates for each population, which would underestimate SIRs if cancer incidence was higher in 1975 than in earlier years; however, a sensitivity analysis restricted to follow-up after 1975 yielded very similar results. It is also possible that some individuals in our study population may harbor GPVs in multiple cancer susceptibility genes, which could further affect cancer risk. To address this potential limitation, we excluded from the analysis 3 families with known GPVs in non-*CDKN2A* cancer susceptibility genes identified through panel testing. However, not all families could be evaluated for GPVs in other genes. Therefore, although unlikely, it is possible that families with GPVs in non-*CDKN2A* cancer susceptibility genes are included in our analysis.

An important strength of our study is that all cancer diagnoses were confirmed using medical records (United States) or cancer registry data (Sweden). However, this could have led to underestimated SIRs for some cancer types if cancer diagnoses could not be confirmed by these methods. To mitigate the impact of multiple comparisons, we applied a Bonferroni correction to determine statistical significance for associations of cancer risk. Because ascertainment bias could also affect results, we performed a sensitivity analysis restricting follow-up time to after the family ascertainment date and found increases in incidence like those from the overall analysis (follow-up starting at birth). A further strength is the inclusion of separate cohorts from different countries. Finally, we also adjusted for family relatedness in the analysis because shared genetic factors and environmental exposures between relatives could affect cancer susceptibility.

Our findings suggest important differences in cancer risk among *CDKN2A* GPV carriers based on transcript alteration, and it will be important for future studies to confirm these results. Interventions aimed at smoking cessation may reduce risk for nonmelanoma cancers, which predominantly occurred in GPV carriers who smoked cigarettes.

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Notes

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Data Availability

The underlying data for this article cannot be shared publicly because it could lead to the identification of patients. Reasonable requests to review the data can be submitted to the corresponding author, and any release of the data would require approval by the NIH Institutional Review Board and Swedish ethical review authority.

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