SHORT REPORT

Prospective risk of cancer in *CDKN2A* germline mutation carriers

A M Goldstein, J P Struewing, M C Fraser, M W Smith, M A Tucker

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Background: The *CDKN2A* gene is the major known high-risk melanoma susceptibility gene. Susceptibility to other cancers has also been suggested. However, most studies examining the risks of other cancers classified individuals according to the family's *CDKN2A* mutation rather than determining individual mutation status. For non-population-based studies, risks could also be biased because of cancer occurrence prior to family ascertainment.

Methods: We examined the risk of non-melanoma cancer in 117 mutation-positive and 136 mutation-negative members from 15 families that had at least two first degree relatives with melanoma and *CDKN2A* mutations restricting the analysis to the period after the families were ascertained (that is, the prospective period) and using individual mutation data. The families have been followed prospectively for 4–26 years starting in the 1970s.

Results: Overall, there was no significant association for mutation-negative subjects (Obs/Exp = 0.3, 95% confidence interval (CI) 0.0 to 1.2) although this group had only two observed cancers. In contrast, mutation-positive subjects had a significantly increased risk for all cancers combined (Obs/Exp = 12/5.5 = 2.2, 95% CI 1.1 to 3.8) primarily because of digestive system tumours, particularly pancreatic cancer. No other organ systems or individual tumour sites showed significantly increased risks.

Conclusions: Differences in *CDKN2A*–non-melanoma cancer associations across studies may result from variation in genetic backgrounds, insufficient follow up, misclassification of mutation carriers, or the presence of other genetic and/or environmental risk factors in both *CDKN2A* mutation carriers and non-carriers. Larger sample sizes, prospective follow up, and individual mutation data will be required to understand these differences.

The *CDKN2A* gene is the major known melanoma susceptibility gene. Germline mutations have been detected in approximately 20% of melanoma-prone families.[1 2] Susceptibility to other cancers has also been suggested. In particular, there is a significantly increased risk of pancreatic cancer in a subset of families with *CDKN2A* mutations.[3–7] And recently, a significantly increased risk of breast cancer was reported in melanoma-prone families with *CDKN2A* mutations from Sweden.[6] However, most studies examining the risks of other cancers have not been population-based because of the difficulties in collecting sufficient numbers of families with mutations. In such situations, the selection and ascertainment methods applied to patients/families may influence the associations observed. Specifically, examination of cancer risks may be subject to bias if cancers that occurred prior to ascertainment of the families, that is, cancer occurrence during the retrospective period, influenced participation or selection of families into a research study and such cancer occurrences were included in the risk assessment. In addition, most studies, including our previous study,[3] classified individuals according to the family's *CDKN2A* mutation rather than determining individual mutation status.[3–7] This approach could have led to misclassification of both mutation-positive and mutation-negative subjects. These complexities, which could produce biased results, require a more refined approach. We, therefore, examined the risk of non-melanoma cancer in melanoma-prone families with *CDKN2A* mutations restricting the analysis to the period after the families were ascertained (that is, the prospective period) and using individual mutation data.

METHODS

Participants and design

Families were recruited for this non-population-based family study if there was a history of invasive melanoma in at least two first degree relatives. The families were referred by health-care professionals or through self-referrals. Written informed consent was obtained prior to participation under an Institutional Review Board-approved protocol. All family members willing to participate in the study were clinically evaluated. Clinical evaluation of family members included complete skin examination and routine medical history. Bloods were collected for genetic studies at the subject's first visit. The subjects for this study were drawn from 15 families in which a *CDKN2A* mutation had been previously identified.[8] All the families were Caucasian and resided in various regions of the United States. Table 1 shows the mutation identified, the number of melanoma patients, the number of subjects with known mutation status, and the date of ascertainment in each family. The families have been followed prospectively for 4–26 years starting in the 1970s. Follow up of the families included periodic clinical evaluation and regular requests for updated medical information including the occurrence of cancer. All cancer diagnoses were confirmed by review of histologic materials, local pathology reports, medical records, or death certificates. Only invasive cancers confirmed by at least one of these methods were included in this study.

Statistical analysis

To estimate the prospective risk of cancer, we calculated numbers of person-years of observation according to sex, age, and the interval from the date of each family's ascertainment to the development of cancer, death, or July 1, 2002. Tumour incidence rates for whites 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

Abbreviations: CI, confidence intervals; SIR, standardised incidence ratios

Table 1 Germline mutations in *CDKN2A* melanoma-prone families

Family	No. patients with melanoma	No. subjects with known *CDKN2A* mutation status	Date of ascertainment of family	Description of *CDKN2A* mutation		
				Exon	Amino acid change	Nucleotide change
A	8	32	1976	1α	1_8dup8	24_47dup24
B	6*	10	1994	1α	L16R	47T>G
C	5	13	1995	2	M53I	159G>C
D	7*	19	1979	2	A58X	172C>T
E	4*	15	1979	2	N71S	212A>G
F	12*	23	1979	2	R87P	260G>C
G	5	12	1979	2	G101W	301G>T
H	3	7	1979	2	G101W	301G>T
I	5*	8	1988	2	G101W	301G>T
J	7	11	1980	2	V126D	377T>A
K	6*	11	1979	2	V126D	377T>A
L	10	33	1998	2	V126D	377T>A
N	8*,*	10	1992	2	S56fs†	167_197del31
P	11	45	1994	2	Chimera‡	240_253del14
Q	3	4	1988	Intron 2	Splicing§	+1g>t

*Includes patient with melanoma in situ; †fs: frameshift mutation; ‡Chimera: 1-80p16:100-133p14; §Splicing mutation results in: R128fs; Ex1α -Ex3delEx2.[9]

occurrences of cancer expected if this group had had the same risk of cancer as the general population. For 1999–2002, 1998 incidence rates were used.[10] Tumours diagnosed before each family was ascertained were excluded, that is, only incident cancers that occurred after ascertainment of each family were included in the analysis. Tests of significance and 95% confidence intervals (CI) for the standardised incidence ratios (the ratio of the number of observed cancer occurrences to the number expected) were calculated exactly on the basis of a Poisson distribution. Standardised incidence ratios (SIR) were calculated separately for subjects who were mutation-positive (with and without melanoma) and mutation-negative. All tests were two-sided.

RESULTS

Only bloodline subjects with known mutation status were included; 84% of bloodline subjects with DNA had been mutation-tested (n = 210) or genotyped to determine if they carried their family's disease-specific haplotype (n = 43). Thus, there were 253 subjects of whom 117 were *CDKN2A* mutation-positive and 136 mutation-negative. Of the 117 mutation-positive subjects, 64 had invasive or in situ melanoma. Of mutation-negative subjects 95% were first degree (n = 76) or second degree (n = 53) relatives of mutation-positive individuals. The remaining seven mutation-negative subjects were third degree relatives of mutation-positive individuals. Table 2A presents the prospective risks of cancers in mutation-positive and mutation-negative subjects. Cancers were considered according to organ system rather than individual site (for one or fewer cancers) because of the relatively small numbers. Table 3 presents details for the 14 reported prospective non-melanoma cancers. Overall, there was no significant association for mutation-negative subjects (SIR = 0.3, 95% CI 0.0 to 1.2), although this group had few observed cancers. In contrast, mutation-positive subjects had a significantly increased risk for all cancers combined (SIR = 2.2, 95% CI 1.1 to 3.8) primarily because of digestive system tumours, particularly pancreatic cancer (table 2A). Four patients with pancreatic cancer from three different families (families F, J, K) were observed (SIR = 38, 95% CI 10 to 97). There were no occurrences of pancreatic cancer in mutation-negative subjects. No other organ systems or individual tumour sites showed significantly increased risks.

Mutation-positive subjects were further split into those with and without invasive or in situ melanoma (table 2B). Nine of the 12 prospective cancers from mutation-positive subjects occurred in patients with melanoma. And as was previously seen, there were significantly increased risks for all cancers combined (SIR = 2.3, 95% CI 1.1 to 4.4), digestive system tumours (SIR = 9, 95% CI 3 to 20), and pancreatic cancer (SIR = 52, 95% CI 14 to 133). The relatively small numbers of cancers, however, yielded imprecise estimates of the prospective cancer risks. Among subjects without melanoma, there were no significant associations; three cancers were observed (SIR = 1.9, 95% CI 0.4 to 5.5), two of which were breast cancer (SIR = 5.5, 95% CI 0.6 to 20.0) from different families (table 3).

Although pancreatic cancer showed a significantly increased risk in mutation-positive subjects, during this prospective follow-up period, only four patients developed pancreatic cancer based on 1500 person-years of observation. In contrast, during the same prospective period, 49 invasive melanomas developed in 22 melanoma patients, including seven patients who developed their first melanoma. In addition, there were 14 deaths related to melanoma during this prospective period. Thus, melanoma remains the major contributor to morbidity and mortality in these subjects. And, although melanoma has a dramatically earlier age at diagnosis in melanoma-prone families with *CDKN2A* mutations,[1 2] the median age at pancreatic cancer diagnosis in this study (70.5 years) was consistent with that observed in the US general population (median 71.0 years).[10]

DISCUSSION

Previous studies have reported significantly increased risks for non-melanoma cancers, especially pancreatic cancer and rarely breast cancer, in *CDKN2A* mutation-positive melanoma-prone families.[1 3–7] Most of these studies, however, did not use mutation data from individual participants and therefore may have misclassified both mutation-positive and mutation-negative subjects.[3–7] In addition, some of these studies were not population-based and so the occurrence of other cancers in the families might have influenced participation or selection of families into a research study. Under this scenario, assessment of cancer risk that included cancers that had occurred in the families prior to ascertainment could have biased the results. To eliminate this potential bias, evaluation of cancer risks from non-population-based family studies should be restricted to the period after ascertainment of the families, if possible. However, this approach requires prospective follow up of the families; such follow up may not be feasible for many study samples. This non-population-based family study tried to eliminate both of these potential problems by restricting the statistical analysis to the

Table 2 Prospective risk of non-melanoma cancer in *CDKN2A* melanoma-prone families

A. *CDKN2A* mutation-positive and mutation-negative subjects								
	Obs	Exp	Obs/Exp	(95% CI)	Obs	Exp	Obs/Exp	(95% CI)
Tumour type	Mutation-positive subjects (1500 person-years)				Mutation-negative subjects (2138 person-years)			
All cancers	12	5.5	2.2	(1.1 to 3.8)	2	6.2	0.3	(0.0 to 1.2)
Digestive system	6	0.9	6.7	(2.4 to 14.6)	0			
Pancreas	4	0.1	38	(10 to 97)	0			
Respiratory system	1	0.8	1.2	(0.0 to 6.5)	0			
Breast	2	0.9	2.2	(0.2 to 8.1)	1	1.2	0.9	(0.0 to 4.8)
Prostate	1	0.8	1.3	(0.0 to 7.4)	0			
Urinary tract	1	0.4	2.6	(0.0 to 14.6)	0			
Brain and CNS	1	0.1	10.6	(0.1 to 59.1)	0			
Lymphatic and haematopoietic	0				1	0.6	1.8	(0.0 to 9.8)
B. *CDKN2A* mutation-positive subjects only								
	Obs	Exp	Obs/Exp	(95% CI)	Obs	Exp	Obs/Exp	(95% CI)
Tumour type	Subjects with melanoma (811 person-years)				Subjects without melanoma (689 person-years)			
All cancers	9	3.9	2.3	(1.1 to 4.4)	3	1.6	1.9	(0.4–5.5)
Digestive system	6	0.7	9.1	(3.3 to 19.8)	0			
Pancreas	4	0.1	52	(13 to 132)	0			
Respiratory system	1	0.7	1.5	(0.0 to 8.5)	0			
Breast	0	0.5		(0.0 to 6.9)	2	0.4	5.5	(0.6–20.0)
Prostate	1	0.6	1.6	(0.0 to 8.8)	0			
Urinary tract	1	0.3	3.4	(0.0 to 19.0)	0			
Brain and CNS	0	0.1		(0.0 to 58.4)	1	0.03	31.9	(0.4–177.3)

CI, confidence interval; Exp, expected; Obs, observed.

prospective time period, that is, after the date of ascertainment, and by using individual mutation data. Although based on small numbers, the results suggested that the major increased risk for non-melanoma cancers in these melanoma-prone families with *CDKN2A* mutations resulted from an increased risk of digestive system tumours, primarily pancreatic cancer. At present, however, we cannot identify the specific genotypes that predispose individuals with a *CDKN2A* mutation to pancreatic cancer.[3–7] And in contrast to the Swedish study of predominantly one single *CDKN2A* founder mutation (113insR),[6] no statistically significantly increased risk for breast cancer was seen here although the trend showed the same direction. Additional studies with much larger sample sizes are required to determine whether specific *CDKN2A* mutations are associated with different types of cancer.

The families for the current study were ascertained through health care professionals or through self-referrals and may not be representative of all melanoma-prone families with *CDKN2A* mutations. In addition, the occurrence of only 14 prospective non-melanoma cancers precluded a more rigorous statistical analysis. All subjects were treated as independent observations and the 11 *CDKN2A* mutations were classified identically. Finally, although not all cancers may have been reported, our regular contact with and follow up of the participants should have limited the chances of missing cancer diagnoses. To further minimise errors, only cancers that could be confirmed through review of histologic materials, local pathology reports, medical records, or death certificates were included in this study.

In summary, evaluation of the prospective risk of cancer in 117 *CDKN2A* mutation-positive participants showed that the major increased non-melanoma cancer risk resulted from digestive system tumours, primarily pancreatic cancer. However, only four patients developed pancreatic cancer based on 1500 person-years of observation compared to 49 prospective invasive melanomas in 22 melanoma patients and 14 deaths related to melanoma during this prospective period. Thus, melanoma remains the major contributor to morbidity and mortality in these subjects. Differences in

Table 3 Prospective non-melanoma cancers in study participants

Subject no.	Tumour type/organ	Histopathology	Age at diagnosis	Family
1	Lung	Squamous carcinoma	78	A
2	Lymphatic/haematopoietic	Multiple myeloma	62	A
3	Prostate	Adenocarcinoma	50	E
4	Brain	Glioblastoma multiforme	60	E
5	Colorectal	Adenocarcinoma	41	F
6	Pancreas	Carcinoma	56	F
7	Colorectal	Adenocarcinoma	38	G
8	Pancreas	Carcinoma	67	J
9	Kidney	Renal cell carcinoma	55	J
10	Pancreas	Adenocarcinoma	76	K
11	Pancreas	Carcinoma	74	K
12	Breast	Infiltrating ductal carcinoma	41	K
13	Breast	Infiltrating ductal and lobular carcinoma	74	K
14	Breast	Infiltrating ductal carcinoma	49	L

CDKN2A–non-melanoma cancer associations across studies may result from bias resulting from inclusion of cancers that occurred during the retrospective period (from non-population-based studies), misclassification of mutation carriers, insufficient/low power, variation in the underlying genetic background of families (for example different *CDKN2A* mutations), or the presence of other genetic and/or environmental risk factors in both *CDKN2A* mutation carriers and non-carriers. Larger numbers of individuals and families with a broad spectrum of mutations, sufficient person-years of prospective follow up, and individual mutation data will be required to better understand differences in these associations.

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Authors' affiliations

A M Goldstein, M C Fraser, M A Tucker, Genetic Epidemiology Branch, Division of Cancer Epidemiology and Genetics, National Cancer Institute, NIH, DHHS, Bethesda, MD 20892, USA
J P Struewing, Laboratory of Population Genetics, Center for Cancer Research, National Cancer Institute, NIH, DHHS, Bethesda, MD 20892, USA
M W Smith, Laboratory of Genomic Diversity, Center for Cancer Research and Basic Research Program, SAIC Frederick, National Cancer Institute at Frederick, NIH, DHHS, Frederick, MD 21702, USA

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Correspondence to: Dr A M Goldstein, Genetic Epidemiology Branch/NCI/NIH/DHHS, Executive Plaza South, Room 7004, 6120 Executive Blvd., MSC 7236, Bethesda, MD 20892-7236, USA; goldstea@exchange.nih.gov

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