**SHORT REPORT**

Prospective risk of cancer in CDKN2A germline mutation carriers

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

**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.

**Background:** The CDKN2A gene is the major known melanoma susceptibility gene. Germline mutations have been detected in approximately 20% of melanoma-prone families. 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. And recently, a significantly increased risk of breast cancer was reported in melanoma-prone families with CDKN2A mutations from Sweden. 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, classified individuals according to the family’s CDKN2A mutation rather than determining individual mutation status. 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 ascertainment (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. 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 observed cancers.

**Abbreviations:** CI, confidence intervals; SIR, standardised incidence ratios
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. 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 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, especially 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, 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).

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. 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. 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
prospective time period, that is, after the date of ascertain-
ment, 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 mela-
noma-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. How-
ever, 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>
<thead>
<tr>
<th>Table 2</th>
<th>Prospective risk of non-melanoma cancer in CDKN2A melanoma-prone families</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. CDKN2A mutation-positive and mutation-negative subjects</td>
<td></td>
</tr>
<tr>
<td>Tumour type</td>
<td>Obs</td>
</tr>
<tr>
<td>All cancers</td>
<td>12</td>
</tr>
<tr>
<td>Digestive system</td>
<td>6</td>
</tr>
<tr>
<td>Pancreas</td>
<td>4</td>
</tr>
<tr>
<td>Respiratory system</td>
<td>1</td>
</tr>
<tr>
<td>Breast</td>
<td>2</td>
</tr>
<tr>
<td>Prostate</td>
<td>1</td>
</tr>
<tr>
<td>Urinary tract</td>
<td>1</td>
</tr>
<tr>
<td>Brain and CNS</td>
<td>1</td>
</tr>
<tr>
<td>Lymphatic and haematopoietic</td>
<td>0</td>
</tr>
</tbody>
</table>

| B. CDKN2A mutation-positive subjects only |
| Tumour type | Obs | Exp | Obs/Exp (95% CI) | Obs | Exp | Obs/Exp (95% CI) |
| 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 | 0 | 0 |
| Pancreas | 4 | 0.1 | 52 (13 to 132) | 0 | 0 | 0 |
| Respiratory system | 1 | 0.7 | 1.5 (0.0 to 8.5) | 0 | 0 | 0 |
| Breast | 0 | 0.5 | 0.0 (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 | 0 | 0 |
| Urinary tract | 1 | 0.3 | 3.4 (0.0 to 19.0) | 0 | 0 | 0 |
| Brain and CNS | 0 | 0.1 | 0.6 (0.0 to 58.4) | 1 | 0.03 | 31.9 (0.4–17.3) |

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

<table>
<thead>
<tr>
<th>Table 3</th>
<th>Prospective non-melanoma cancers in study participants</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subject no.</td>
<td>Tumour type/organ</td>
</tr>
<tr>
<td>1</td>
<td>Lung</td>
</tr>
<tr>
<td>2</td>
<td>Lymphatic/haematopoietic</td>
</tr>
<tr>
<td>3</td>
<td>Prostate</td>
</tr>
<tr>
<td>4</td>
<td>Brain</td>
</tr>
<tr>
<td>5</td>
<td>Colorectal</td>
</tr>
<tr>
<td>6</td>
<td>Pancreas</td>
</tr>
<tr>
<td>7</td>
<td>Colorectal</td>
</tr>
<tr>
<td>8</td>
<td>Pancreas</td>
</tr>
<tr>
<td>9</td>
<td>Kidney</td>
</tr>
<tr>
<td>10</td>
<td>Pancreas</td>
</tr>
<tr>
<td>11</td>
<td>Pancreas</td>
</tr>
<tr>
<td>12</td>
<td>Breast</td>
</tr>
<tr>
<td>13</td>
<td>Breast</td>
</tr>
<tr>
<td>14</td>
<td>Breast</td>
</tr>
</tbody>
</table>
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.

ACKNOWLEDGEMENTS
We are indebted to the participating families, whose generosity and cooperation have made this study possible. The authors also wish to acknowledge the contributions to this work that were made by Laura Fontaine, BSN, Virginia Pichler, RN PhD, and Deborah Zametkin, MSN. We would like to thank Melissa Levasseur for genotyping some of the samples used in this work and Joe Barker, IMS, for analytic support.

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
This publication has been funded in part with funds from the National Cancer Institute, NIH, DHHS, Contract No. NO1-CO-12400.
Conflict of interest: none declared.

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

Revised version received 8 March 2004
Accepted for publication 9 March 2004

REFERENCES
9 Rutter JL, Goldstein AM, Davila MR, Tucker MA, Struewing JP. CDKN2A point mutations (D153spc.453c.45Gc.45T) and V52+1G>T result in aberrant splice products affecting both p16\(^{\text{NK4A}}\) and p14\(^{\text{ARF}}\). Oncogene 2003;22:4444–8.
Prospective risk of cancer in CDKN2A germline mutation carriers

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

J Med Genet 2004 41: 421-424
doi: 10.1136/jmg.2004.019349

Updated information and services can be found at:
http://jmg.bmj.com/content/41/6/421

These include:

References
This article cites 8 articles, 3 of which you can access for free at:
http://jmg.bmj.com/content/41/6/421#BIBL

Email alerting service
Receive free email alerts when new articles cite this article. Sign up in the box at the top right corner of the online article.

Topic Collections
Articles on similar topics can be found in the following collections

- Cancer: dermatological (43)
- Dermatology (240)
- Pancreas and biliary tract (110)

Notes

To request permissions go to:
http://group.bmj.com/group/rights-licensing/permissions

To order reprints go to:
http://journals.bmj.com/cgi/reprintform

To subscribe to BMJ go to:
http://group.bmj.com/subscribe/