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Melanoma risk for *CDKN2A* mutation carriers who are relatives of population-based case carriers in Australia and the United Kingdom

Anne E Cust[1], **Mark Harland**[2], **Enes Makalic**[1], **Daniel Schmidt**[1], **James G Dowty**[1], **Joanne F Aitken**[3], **Chantelle Agha-Hamilton**[4], **Bruce K Armstrong**[5], **Jenny H Barrett**[2], **May Chan**[2], **Yu-Mei Chang**[2], **Joanne Gascoyne**[2], **Graham G Giles**[1,6], **Elizabeth A Holland**[4], **Richard F Kefford**[4], **Kairen Kukalizch**[2], **Johanna Lowery**[7], **Juliette A Randerson-Moor**[2], **Helen Schmid**[4], **Claire F Taylor**[8], **Linda Whitaker**[2], **John L Hopper**[1], **Julia A Newton-Bishop**[2], **Graham J Mann**[4], **D Timothy Bishop**[2], **Mark A Jenkins**[1]

[1]Centre for Molecular, Environmental, Genetic and Analytic (MEGA) Epidemiology, Melbourne School of Population Health, The University of Melbourne, Melbourne, Australia

[2]Section of Epidemiology and Biostatistics, Leeds Institute of Molecular Medicine (LIMM), University of Leeds, Leeds, United Kingdom

[3]Viertel Centre for Research in Cancer Control, Cancer Council Queensland, Brisbane, Australia

[4]Westmead Institute for Cancer Research, The University of Sydney at Westmead Millennium Institute and Melanoma Institute Australia, Sydney, Australia

[5]Sydney School of Public Health, The University of Sydney, Sydney, Australia

[6]Cancer Epidemiology Centre, Cancer Council Victoria, Melbourne, Australia

[7]Mutation Detection Facility, Cancer Research UK, St James's University Hospital, Leeds, United Kingdom

[8]Genomics Facility, Leeds Cancer Research UK Centre, University of Leeds, Leeds, United Kingdom

Abstract

Background: *CDKN2A* mutations confer a substantial risk of cutaneous melanoma, however the magnitude of the risk is uncertain.

Methods: We estimated the hazard ratio (HR) and the average age-specific cumulative risk (i.e. penetrance) of reported melanoma for *CDKN2A* mutation carriers in case families using a

Correspondence to: Dr Anne Cust, Centre for Molecular, Environmental, Genetic & Analytic Epidemiology, School of Population Health, Level 1, 723 Swanston Street, The University of Melbourne, VIC 3010 Australia, T: +61 3 8344 0863; F: +61 3 9349 5815 ; aecust@unimelb.edu.au.

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modified segregation analysis of the first- and higher-degree relatives of 35 population-based cases. The study sample included 223 relatives of 13 early-onset melanoma cases (aged 18-39 years at diagnosis) from Melbourne, Sydney and Brisbane, Australia, and 322 relatives of 22 melanoma cases diagnosed at any age from Yorkshire, United Kingdom (UK).

Results: The estimated HR for melanoma for mutation carriers relative to the general population decreased with regions of increasing ambient ultraviolet irradiance: being higher for the UK than Australia (87, 95% confidence interval (CI) 50-153 versus 31, 95% CI 20-50, P=0.008), and in Australia, 49 (95% CI 24-98) for Melbourne, 44 (95% CI 22-88) for Sydney, and 9 (95% CI 2-33) for Brisbane (P=0.02). Penetrance did not statistically significantly differ between the two countries or across regions within Australia. For example, we estimated that 16% (95% CI 10%-27%) of UK and 20% (95% CI 13%-30%) of Australian *CDKN2A* mutation carriers would be diagnosed with melanoma by age 50 years, and 45% (95% CI 29%-65%) and 52% (95% CI 37%-69%), respectively, by age 80 years.

Conclusions: *CDKN2A* mutation carriers living in geographic regions with different levels of ambient UV irradiance appear to have the same cumulative risk of melanoma.

Keywords

melanoma; penetrance; *CDKN2A*; risk; genetic susceptibility

INTRODUCTION

CDKN2A on chromosome 9p21 is the major known melanoma susceptibility gene,[1, 2] encoding two distinct proteins, p16INK4A and p14ARF, which are involved in cell cycle control, tumour suppression and melanocyte senescence.[1] The p16INK4A protein binds to the cyclin-dependent kinases CDK4 and CDK6, inhibiting phosphorylation of the retinoblastoma protein and progression of the cell through the G1 cell cycle checkpoint. The p14ARF protein induces cell cycle arrest or apoptosis via the p53 pathway.

Carriers of a *CDKN2A* mutation have a substantial risk of developing cutaneous malignant melanoma, however the absolute and relative magnitudes of the risk are uncertain. Two international studies[3, 4] have reported apparently different estimates of age-specific cumulative risk (i.e. penetrance). The Melanoma Genetics Consortium (GenoMEL) study of 80 multiple-case families ascertained predominantly from family cancer clinics estimated that 30% (95% CI: 12% to 62%) of mutation carriers would develop melanoma by age 50 years and 67% (95% CI: 31% to 96%) by age 80 years, with risk varying by geographical region, being higher in Australia (91% by age 80 years) than the United States (76% by age 80 years) and Europe (58% by age 80 years).[4] On the other hand, the Genes, Environment and Melanoma (GEM) study, using data on first-degree relatives of 65 population-based melanoma carriers (probands), estimated penetrance of 14% (95% CI = 8% to 22%) by age 50 years and 28% (95% CI: 18% to 40%) by age 80 years, and no significant differences by region.[3] They also found that the penetrance was lower when estimated from carrier families ascertained through single primary melanoma carrier probands than through multiple primary melanoma carrier probands (P=0.04).

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Estimates of penetrance derived from carrier families identified because they have multiple cases of melanoma through clinic-based sampling (as in the GenoMEL study) could be higher than when derived from carriers identified independently of their family history through population-based sampling of cases (as in the GEM study) because carrier families could differ in the prevalence of other genetic or environmental risk factors or the severity of the mutations. That is, penetrance could depend on the context or setting, so there is no single penetrance.[5] For each study, the penetrance being estimated is an average over the mutations causing disease in the probands and the other factors operating in their families.[5, 6, 7]

To provide further insights into penetrance, we estimated the age-specific cumulative risk and hazard ratio (relative risk) of melanoma for carriers of deleterious mutations in *CDKN2A* by studying the relatives of population-based early-onset melanoma cases (aged 18-39 years at diagnosis) from three major cities in Australia and of population-based melanoma cases diagnosed at any age from Yorkshire, United Kingdom (UK).

METHODS

Subjects

The study sample comprised 35 population-based families (13 from Australia and 22 from the UK) in which probands had histopathologically-confirmed invasive cutaneous melanoma and had been found from mutation screening to carry a pathogenic *CDKN2A* mutation. Families were obtained from two sources: 1) the Australian Melanoma Family Study,[8] a multi-centre, population-based, case-control-family study of early-onset melanoma; and 2) the Leeds case-control study,[9, 10] a population-based, case-control study of melanoma in the UK. Ethics committee approval for both studies was obtained from all relevant ethics committees in each country, and all participants gave written informed consent.

Australian Melanoma Family Study

The study sample, recruitment methods, data collection and subject characteristics have been described in detail.[8] Briefly, probands were diagnosed between 1st July 2000 and 31st December 2002 at ages 18-39 years with incident, histopathologically-confirmed, first-primary invasive cutaneous melanoma. At the time of recruitment, probands were living in the greater urban areas of Brisbane (27.3°S, high ambient UV), Sydney (33.6°S, intermediate ambient UV) or Melbourne (37.5°S, lower ambient UV), the three largest urban populations in Australia.[8] Probands were identified from population-based state cancer registries; participation was 54% of those eligible and 76% of those contactable. Of the 629 case probands recruited, 596 (95%) gave a blood sample and were screened for mutations in *CDKN2A*.

First- and second-degree relatives were recruited where possible via the probands. One mutation-carrying proband was excluded from this analysis because no information could be collected for their relatives. The Cannings-Thompson sequential ascertainment scheme[11] was used to extend recruitment to higher-degree relatives in an unbiased way, such that any additional diagnosis of melanoma (either *in situ* or invasive) for any eligible relative

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subsequently made eligible all first-degree relatives of the newly identified melanoma case. A blood sample and information on lifetime sun exposure, phenotype and residence history were requested from probands and participating living relatives, and proxy-reported demographic and cancer information was collected for deceased relatives. Verification of reported melanomas in relatives was sought from cancer registries, hospital and pathology records, treating clinicians, general practitioners and death certificates after participant or next-of-kin (if participant deceased) consent had been obtained. For the relatives in this analysis, 48% of melanoma reports were confirmed.

Leeds case-control study

The study sample and methods have been previously described.[9, 10] Briefly, between September 2000 and December 2006, 1043 probands diagnosed between 18 and 82 years with incident, histopathologically-confirmed, invasive cutaneous melanoma living in a geographically defined area of Yorkshire and the Northern region of the UK (53.8° N) were recruited. Proband participation was 67%. Between September 2000 and June 2003 all patients with invasive melanoma were invited to participate, but from July 2003 to December 2006 patients with Breslow thickness less than 0.75mm were not invited. The cases were identified through clinicians, pathology registers and the Northern and Yorkshire cancer registry to ensure maximal ascertainment. First- and second-degree relatives of probands, and higher-degree relatives with complete ascertainment were included irrespective of melanoma status. Probands were asked to report their personal and family history of melanoma. Melanomas were verified by obtaining histopathology reports where possible or by Cancer Registry for probands and where relatives were able or willing to consent to this process. In the absence of consent to confirm a relative's melanoma then the best judgement of the interviewers was reported. For the relatives in this analysis, 31% of melanoma reports were confirmed. Germline DNA was screened for mutations in *CDKN2A* for the first 1000 probands.

Screening for *CDKN2A* mutations

Probands were screened for variants in the *CDKN2A* coding region, including exons 1α, 1β, 2, and 3, splice junctions, and the 5′ and 3′ UTRs. Mutation screening was also conducted for exon 2 (the p16INK4A binding domain) of *CDK4*. All available samples in relatives of the identified pathogenic mutation carriers (see below) were then screened for the mutation identified in the proband. Australian samples were screened at the University of Sydney, using bidirectional DNA sequencing (*CDKN2A* exons 1α, 1β, 3 and *CDK4* exon 2) and denaturing high performance liquid chromatography (DHPLC) (*CDKN2A* exon 2 only). UK samples were screened at St James's University Hospital, Leeds, using either DHPLC or high resolution melting curve analysis (HRMCA). Primers, protocols and reaction conditions were as previously described for DHPLC[12] and HRMCA[13, 14] These methods for identifying mutations has been shown to be equivalent.[12] All amplicons that displayed an aberrant SSCP, DHPLC or HRMCA profile were bi-directionally sequenced to identify the underlying nucleotide change, as previously described.[12]

Variants were designated as pathogenic if they had been previously reported to cosegregate with melanoma in multiple-case kindreds, if there was functional evidence of impaired

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protein function, or if these data were not available, if the mutation satisfied bioinformatic criteria (SIFT, http://blocks.fhcrc.org/sift/SIFT.html; Polyphen, http://genetics.bwh.harvard.edu/pph/; pMut, http://mmb2.pcb.ub.es:8080/PMut/) predicting loss of function and the variants were never or rarely observed in previous reports of control series (Harland et al, in preparation).

Statistical analysis

Hazard ratio (HR; the ratio of age-specific incidence of melanoma for mutation carriers to that for non-carriers) and age-specific cumulative risk (penetrance) were estimated from data on carrier families using modified segregation analysis. This method incorporates both genotyped and ungenotyped relatives and conditions on ascertainment to produce unbiased estimates. Models were fitted and statistical inference conducted under maximum likelihood theory using MENDEL version 3.2.[15] The joint log-likelihood was calculated for each carrier family, based on information for each member on their observed melanoma status, age at last contact, death or diagnosis, family relationships and carrier status (carrier, non-carrier or unknown). To adjust for ascertainment, the joint likelihood for each pedigree was conditioned on the proband's melanoma status, mutation status and age at diagnosis. The log HR, and hence the age-specific cumulative risk, was estimated by maximizing the sum of the logs of the conditioned likelihoods, assuming families were independent. Likelihood ratio tests were used to test whether the HR and penetrance estimates differed by sex, region or age.

The population *CDKN2A* allele frequency was assumed to be 0.001 and mutations were assumed to act on disease risk in a dominant fashion. As *CDKN2A* mutations are rare, we assumed the incidence of melanoma in non-carriers was equal to that for the general population. Average sex- and age-specific population incidences of melanoma were obtained from 'Cancer Incidence in Five Continents'[16] for each geographic region from which probands were recruited (Queensland, New South Wales, and Victoria in Australia, and Yorkshire in the UK). Population incidences were selected for the period corresponding to the relatives' average year of melanoma diagnosis (1980 for Australia, 1994 for the UK); thus for Australia volume 5 (1978-1982) was used and for the UK volume 8 (1993-1997) was used.[16] Region and sex-specific HR and penetrance estimates were estimated using region and sex-specific population melanoma incidences as the baseline. To derive estimates of penetrance for all Australian carriers combined, we constructed an average population incidence curve using the incidence curves for each state and sex, weighted in proportion to the number of probands and relatives that were male and female recruited in that state. Similarly, to derive estimates of penetrance for UK males and females combined, we constructed an average population incidence curve using the incidence curves for each sex, weighted in proportion to the number of males and females recruited in the UK. Ten-year cumulative risk estimates of melanoma for *CDKN2A* mutation carriers who have no melanoma at the beginning of the 10-year period were calculated as $[R(t + 10) - R(t)]/[(1 - R(t)]$, where t is the carrier's age in years and $R(t)$ and $R(t + 10)$ are the relevant cumulative risks to ages t and $t + 10$ years, respectively. Confidence intervals for the 10-year estimates were calculated in the same way, using the lower and upper bounds of the 95% confidence intervals of the cumulative risks.

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Age was recorded at diagnosis of first melanoma, death, or last known age, whichever occurred first. For relatives known to be alive but missing a date of last contact, we assigned the date of proband interview (Australia) or the 1st January 2005 (UK). Relatives whose age was unknown were assigned an age of 0 years and therefore did not contribute to penetrance estimates. All confirmed and unconfirmed reports of melanomas for relatives were included in the analysis.

To test the robustness of our assumptions we conducted several sensitivity analyses: 1) using imputed ages where possible for relatives with missing ages; 2) including confirmed melanomas only and assuming that those with an unconfirmed report of melanoma did not have melanoma; 3) using more recent population incidences of melanoma from 'Cancer Incidence in Five Continents' volume 9, 1998-2002;[17] and 4) using a population allele frequency of 0.0001. For 1) we used the following methods to impute missing ages: for relatives missing a date of birth, the year of birth was assigned in the following order, as either i) the average known birth year of siblings if available, or ii) as 20 years (women) or 22 years (men) earlier than their first offspring's year of birth, or iii) based on the spouse's year of birth, assuming the wife was two years younger than the husband. If none of the above were available then the relative was assigned age 0. For relatives assigned a year of birth and assumed to be alive at the time of the proband's interview, age was calculated as year of proband interview (Australia) or the 1st January 2005 (UK) minus assigned year of birth, and age was censored at age 80 years.

To estimate the number of mutation carriers in the identified carrier families, we calculated the probability of being a *CDKN2A* mutation carrier for each ungenotyped relative, based on the genetic relatedness to known or inferred carriers and non-carriers (but ignoring melanoma status). This calculation assumed Mendel's laws of inheritance and the population allele frequency noted above, and was conducted by modifying MENDEL 3.2. The total number of mutation carriers was estimated by summing the carrier probabilities of the ungenotyped relatives and adding this to the number of known carriers.

RESULTS

A total of 20 different *CDKN2A* pathogenic mutations were identified in 35 probands. These mutations and their predicted effects on the p16INK4A and p14ARF protein coding sequences are presented in Table 1. The mutations included three small exonic insertions or deletions affecting p16INK4A only found in seven probands, seven missense mutations predicted to affect p16INK4A only found in nine probands, three missense mutations affecting p14ARF only found in three probands, one small exonic deletion affecting both p16INK4A and p14ARF found in two probands, four missense mutations affecting both p16INK4A and p14ARF found in seven probands, and two mutations outside the coding region (c.−34G>T false start mutation affecting p16INK4A only, and the intronic c.458-105A>G mutation likely affecting both p16INK4A and p14ARF) found in seven probands. In addition, a nonsense mutation p.W34X was identified in exon 1β of one proband, but was excluded from this analysis because no information could be collected for the proband's relatives. No mutations were identified in *CDKN2A* exon 3 or *CDK4* exon 2.

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A description of the Australian and UK carrier families are shown in Table 2. In the 13 Australian carrier families, 17 (52%) of 33 genotyped relatives (32 first-degree, one second-degree) were found to be *CDKN2A* mutation carriers. In the 22 UK carrier families, 10 (56%) of 18 genotyped relatives (17 first-degree, one third-degree) were found to be *CDKN2A* mutation carriers. We estimated a total of 72 mutation carriers in the Australian carrier families and 115 mutation carriers in the UK carrier families.

Table 3 shows, for descriptive purposes, the characteristics of all 35 probands and the 419 relatives who either were known to carry a *CDKN2A* mutation or had an estimated 25% or higher probability of being a mutation carrier. Although all relatives were included in the analyses, regardless of their probability of carrying a mutation, relatives whose probability of being a mutation carrier was less than 25% are not shown in Table 3 because of their minor influence on the penetrance estimates in comparison with known or more likely mutation carriers. The mean age at diagnosis of melanoma was 34 years for Australian probands (reflecting the recruitment criteria of early-onset melanoma) and 47 years for UK probands (who were unselected for age at diagnosis). Affected relatives who were known or likely carriers in the Australian and UK carrier families had a similar mean age at diagnosis of melanoma (47 years).

Estimates of HRs and cumulative risk of melanoma for *CDKN2A* mutation carriers are shown in Table 4. The incidence of melanoma for carriers of a *CDKN2A* mutation relative to the general population was higher in the UK (HR 87, 95% CI 50-153) than in Australia (HR 31, 95% CI 20-50), P for heterogeneity (P_{het})=0.008. There was modest evidence that the HR for melanoma was greater for female carriers than male carriers in Australia (HR = 48 and 18, respectively, P_{het}=0.07) but not in the UK (HR = 78 for females and 98 for males, P_{het}=0.20). The HRs for Australian carriers differed by geographical region (P_{het}=0.02) with the HR for melanoma being lowest for carrier families based in Brisbane (HR 9, 95% CI 2-33), the most northerly city of recruitment (i.e. high ambient ultraviolet irradiance), and the HR highest for those based in Melbourne (HR 49, 95% CI 24-98), the most southerly. There was no evidence that the HR differed by age of the carrier (P_{het}=0.9).

We estimated that 45% (95% CI 29%-65%) of UK and 52% (95% CI 37%-69%) of Australian *CDKN2A* mutation carriers would be diagnosed with melanoma by age 80 years (Table 4). Cumulative risk estimates are shown separately for males and females and for different geographic regions (Table 4 and Figure 1). Penetrance did not statistically significantly differ by sex or geographic region. By age 80 years, the estimated proportion of mutation carriers that would be diagnosed with melanoma was 64% (95% CI 42%-85%) for Australian females, 38% (95% CI 19%-66%) for Australian males, 45% (95% CI 23%-75%) for UK females, and 45% (95% CI 24%-73%) for UK males. For a 60-year old *CDKN2A* mutation carrier without a previous melanoma diagnosis, the 10-year risk of melanoma was estimated to be 16% (95% CI 10%-24%) in Australia, and 12% (7%-20%) in the UK (Table 5).

Several sensitivity analyses were conducted. We were able to impute ages for 16 of the 20 Australian relatives whose ages were not known, and for 111 of the 170 UK relatives whose ages were not known. When using imputed ages, the melanoma risk estimates for Australian

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carriers were very similar to that based on known ages only (HR, and penetrance at age 80, respectively, for Brisbane = 9, 28%; Sydney = 42, 55%; Melbourne = 49, 58%), but for UK carriers were lower than that based on known ages (HR = 46, 95% CI 26-81, and penetrance at age 80 = 27%, 95% CI 16%-43%). When only confirmed reports of melanoma in relatives were considered as affected, the melanoma risk estimates were approximately halved for Australian carriers (HR = 14, 95% CI 7-27; penetrance at age 80 = 28%, 95% CI 16%-47%) and were two-thirds lower for UK carriers (HR= 23, 95% CI 8-65; penetrance at age 80 = 15%, 95% CI 5%-36%). The estimated HRs were also lower when we used the more recent (higher) population incidences of melanoma rather than the incidences corresponding to relatives' average melanoma diagnosis dates, although this resulted in minimal change to penetrance estimates for Brisbane and UK carriers, and slightly higher penetrance estimates for Sydney and Melbourne carriers (results not shown). Changing the *CDKN2A* allele frequency from 0.001 to 0.0001 had minimal influence on the HR or penetrance estimates (results not shown).

DISCUSSION

Our results are based on *CDKN2A* mutation carriers who are relatives of population-based melanoma cases from Australia and the UK who were unselected for family history. We estimated that the relative increase in the risk of melanoma for *CDKN2A* mutation carriers in this context was higher for carriers living in the UK (87 times that of the general population) than for carriers living in Australia (31 times that of the general population), and in Australia the relative risk of melanoma for mutation carriers differed by region and marginally by sex. However, there was no evidence that the cumulative risk estimates were different between Australia and the UK, with 20% of Australian and 16% of UK *CDKN2A* mutation carriers estimated to be diagnosed with melanoma by age 50 years, and 52% and 45%, respectively, by age 80 years.

Our findings suggest that ambient UV irradiance might not further increase cumulative risk of melanoma for *CDKN2A* mutation carriers as the penetrance was similar whether living in Australia or the UK. Similarly, in Australia, the penetrance was not higher for carriers in Brisbane, the city with the highest ambient UV irradiance. In previous studies, the GenoMEL penetrance estimates differed substantially by geographical location, and were highest in regions with the highest population melanoma incidence,[4] whereas the GEM penetrance estimates did not differ by country.[3]

In Australia, the risk of melanoma was marginally higher for female carriers than for male carriers, but there was no evidence of any sex differences in the UK. The GenoMEL study observed a 24% lower penetrance of melanoma for male carriers than female carriers although this difference was not statistically significant.[4] Although these sex differences might reflect the small numbers in these subgroups, it is possible that there is a more complex association between *CDKN2A* mutations, sun exposure, and sex.

Given the similar penetrances by region, the differences in the relative risk of melanoma for carriers compared with non-carriers that we observed between regions reflects differences in the baseline age-standardised melanoma incidences, with the relative risk increasing as the

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level of ambient UV irradiance decreases. Our relative risk estimates of melanoma (87 in the UK and 31 in Australia) for *CDKN2A* mutation carriers are similar to a study of 13 multiple-case families from the United States,[18] which reported a relative risk of melanoma of 57 (95% CI 17-259) for *CDKN2A* mutation carriers compared with non-carriers. A much lower relative risk of 4.3 (95% CI 2.3-7.7) was observed by the GEM study,[19] but this was for relative risk of subsequent melanoma for *CDKN2A* mutation carriers compared with those without a *CDKN2A* mutation among individuals already diagnosed with a single primary melanoma, who are known to be at higher baseline risk than the general population.

Penetrance estimates of melanoma for carriers of a *CDKN2A* mutation in the population-based GEM study were 14% by age 50 years and 28% by age 80 years,[3] and in the multiple-case, predominantly clinic-based GenoMEL study were 30% by age 50 years and 67% by age 80 years.[4] Our penetrance estimates sit roughly in between these two previous studies. There are several reasons why risk estimates might differ between studies. First, they may depend on the populations studied, and on characteristics of the study sample and the selection criteria; for example, there may be other genetic or environmental risk factors shared by family members with multiple cases. This rationale is supported by findings from the GEM study, in which the penetrance estimated from the families of carrier probands with multiple primary melanoma was higher than that for families of carrier probands with single primary melanoma.[3] In our study, the Australian and UK study populations also differed in that all of the Australian probands had early-onset melanoma (aged 18-39 years at diagnosis) whereas the UK probands were unselected for age at diagnosis. Differences in age at diagnosis might be related to the type of mutation or to the presence of other melanoma risk factors, and it is possible that risk estimates derived from families with early-onset melanoma might be higher than from families with melanoma diagnosed at any age. In addition, risk estimates derived from population-based sources including our study might still be higher than for mutation carriers in the general population without a family history of melanoma, since all relatives in our study had at least one relative (the proband) diagnosed with melanoma.

Second, there is some evidence that different mutations on the *CDKN2A* gene confer different risks of melanoma.[4, 19] In the GenoMEL study, individuals with mutations that affect both p16INK4A and p14ARF had a risk of melanoma 1.8 times that of carriers of a mutation affecting p16INK4A only.[4] In the GEM study, missense mutations affecting both p16INK4A and p14ARF and the non-coding mutation at c.−34G>T conferred statistically significantly greater relative risk of melanoma than mutations that were insertions or deletions or affecting only p16INK4A or only p14ARF.[19] In our study, nine (26%) of the 35 families had missense mutations affecting both p16INK4a and p14ARF, or the c.−34G>T mutation, compared to 40% of mutations in the GEM study and 41% of mutations in the GenoMEL study. Furthermore, neither the GEM nor GenoMEL studies genotyped exon 1β, which is unique to p14ARF, and in which 3 missense mutations were found in three (9%) families. The GEM study also did not screen for the deep intronic c.458-105A>G mutation that was present in 5 (14%) of the families described here. Also in contrast to the GEM and GenoMEL studies, we designated the c.373G>C variant as non-causal, due to its presence in control probands and lack of cosegregation in a number of melanoma pedigrees.

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Each study has also used a different statistical method. An advantage of the modified segregation analysis used here is that this method maximizes power by incorporating data on ungenotyped as well as genotyped relatives, by considering all possible genotype combinations and, essentially, weighting them by their likelihood of occurring. Another advantage is that the method incorporates a rigorous method of adjusting for ascertainment, thereby reducing bias.

This study is supported by a robust population-based design in which we systematically recruited first and higher-degree relatives of probands who were unselected for family history. We also attempted to verify reports of melanoma for relatives, in contrast to the GEM study.[3] The melanoma risk estimates were lower when confined to confirmed reports of melanoma than when all reported melanomas were included. The true risk estimates likely lie between the two, since accuracy of self-reports of melanoma is relatively poor compared with other cancers, especially in Australia[20, 21] however a proportion of reports unable to be verified would be genuine. There was a lack of statistical power to examine the associations with melanoma risk for different types of *CDKN2A* mutations or to examine the influence of phenotype (e.g. number of nevi) or personal sun exposure on the risk estimates. It has been shown that MC1R variants, pigmentation and nevus phenotype influence melanoma risk for *CDKN2A* carriers.[22] Another potential limitation was that about half of the UK relatives and 9% of the Australian relatives had an unknown age and thus were not included in the main penetrance analysis. The lower risk estimates for the UK from the sensitivity analysis that included imputed ages suggests two possibilities. On the one hand, if melanoma was under-reported in those with missing ages but the true incidence was equal to that of relatives with known ages then our main estimate would be unbiased for UK carriers. On the other hand, if recording of age information was less diligent for unaffected relatives, then our main estimate of penetrance may biased upwards for UK carriers.

This study provides refinement of information on age-specific cumulative risk and relative risk of melanoma for individuals with a *CDKN2A* mutation living in sunny or cooler regions, which will be useful to genetic counsellors and clinicians. These risk estimates are most relevant to mutation carriers with a weak family history of melanoma.

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Abbreviations:

AMFS	Australian Melanoma Family Study

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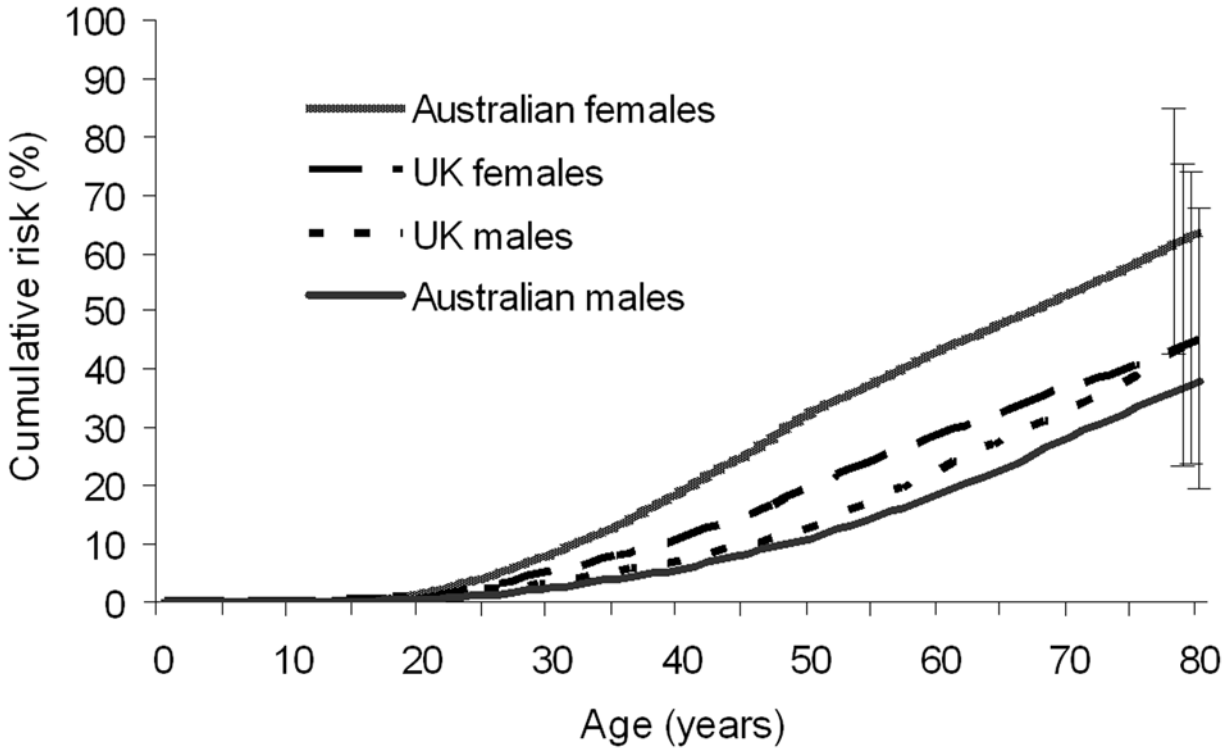

Figure 1. Age-specific cumulative risk of melanoma for carriers of a *CDKN2A* mutation, by country and sex.

The bars at age 80 indicate the 95% confidence intervals for the penetrance at age 80 for each of the four groups.

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Table 1.

CDKN2A mutations identified in population-based families in Australia and the United Kingdom

Gene element	CDK2NA gene variant (protein variant)				Families by Region			
	$p16^{INK4A}$		$p14^{ARF}$		Australia			UK
	Nucleotide change[1]	Effect on protein	Nucleotide change[1]	Effect on protein[2]	Sydney	Melbourne	Brisbane	Leeds
5' UTR	c.−34G>T	r.−34_−32>p.M1	_		1			1
Exon 1α	c.9_32del24	p.A4_10Edel7	_					1
	c.32_33ins9_32	p.M1_S8dup	_		1			3
	c.52_57dup6	p.20T_21Adup	_			1		1
	c.68G>A	p.G23D	_					1
	c.95T>C	p.L32P	_			1		
	c.104G>C	p.G35A	_					1
Exon 1β	_		c.81C>G	p.I27M		1		
	_		c.193G>A	p.G65S			1	
	_		c.193G>C	p.G65R	1			
Exon 2	c.159G>C	p.M53I	c.202G>C	p.D68H				4
	c.188T>C	p.L63P	c.231T>C	p.A77A (silent)				1
	c.194T>C	p.L65P	c.237T>C	p.A79A (silent)			1	
	c.202_203GC>TT	p.A68L	c.245_246GC>TT	p.R82L	1			
	c.206A>G	p.E69G	c.249A>G	p.G83G (silent)	1		2	
	c.212A>C	p.N71T	c.255A>C	p.Q85H				1
	c.228_246del19	p.L77TfsX62	c.271_289del19	p.R90VfsX75				2
	c.251A>C	p.D84A	c.294A>C	p.R84R (silent)				1
	c.331G>A	p.G111S	c.374G>A	p.G125E				1
Intron 2	c.458-105A>G	altered splicing	_	altered splicing		1		4
Total					5	4	4	22

[1] Nucleotides are numbered from the first A of the initiation codon of $p16^{INK4A}$ in the standard nomenclature for mutations employed.[23]

[2] Synonymous variants are presumed ‘silent’.

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Table 2.

Characteristics of population-based families carrying *CDKN2A* mutations, in Australia and the United Kingdom

Country	Number of families	Number of probands' relatives				Relatives, % male	Relatives with melanoma[1]	Family history of melanoma for proband			
								Number (%) of relatives with melanoma in each family			
		Total (average per proband)	1st degree	2nd degree	3rd or higher degree			0	1	2	≥ 3
United Kingdom	22	322 (14.6)	122 (38%)	193 (60%)	7 (2%)	56%	16 (5%)	14 (64%)	2 (9%)	4 (18%)	2 (9%)
Australia	13	223 (17.2)	67 (30%)	136 (61%)	20 (9%)	54%	21 (9%)	3 (23%)	5 (38%)	3 (23%)	2 (15%)

[1] Includes confirmed and unconfirmed reports of melanoma in relatives

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Table 3.

Characteristics of probands and relatives who are known carriers of a *CDKN2A* mutation or with a predicted 25% or higher probability[1] of being a carrier, by region of the proband and sex

Geographic region and sex	Number of families	Number of relatives with ≥ 25% probability of being a carrier[1]	Mean age (range) at diagnosis of melanoma of proband	Mean last known age (range) of relatives without melanoma[2]	Number (%) of relatives with melanoma[2]	Mean age (range) at diagnosis of melanoma[2] in relatives
United Kingdom						
All	22	276	47 (24-72)	50 (0-97)	13 (5)	47 (17-75)
Females	-	119	46 (24-72)	49 (0-97)	6 (5)	43 (17-66)
Males	-	157	50 (41-67)	50 (6-88)	7 (4)	51 (39-75)
Australia						
All	13	143	34 (27-40)	57 (18-91)	20 (14)	47 (18-87)
Females	-	69	31 (27-37)	62 (26-91)	13 (19)	46 (18-84)
Males	-	74	36 (31-40)	53 (18-86)	7 (9)	50 (22-87)
Brisbane	4	41	31 (27-36)	61 (26-91)	3 (7)	48 (22-67)
Sydney	5	45	35 (29-40)	58 (18-85)	9 (20)	49 (18-87)
Melbourne	4	57	34 (30-39)	55 (25-86)	8 (14)	45 (27-77)

[1] Carrier probability was calculated from the genetic relatedness to known or inferred carriers, and not from melanoma status. The calculation assumes Mendel's laws of inheritance, Hardy-Weinberg equilibrium and a population allele frequency of 0.001. Excludes 4 UK individuals whose sex was unknown.

[2] Includes confirmed and unconfirmed reports of melanoma in relatives

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Table 4.

Estimates of hazard ratios and age-specific cumulative risk of melanoma from birth, for carriers of a *CDKN2A* mutation, by region and sex

		Age-specific cumulative risk % (95% CI) for mutation carriers				
Geographic region and sex	**Hazard ratio[1] (95% CI)**	**To age 40**	**To age 50**	**To age 60**	**To age 70**	**To age 80**
United Kingdom						
All	87 (50-153)	9 (5-15)	16 (10-27)	26 (16-41)	35 (22-53)	45 (29-65)
Females	78 (33-181)	11 (5-23)	20 (9-40)	29 (14-55)	37 (18-66)	45 (23-75)
Males	98 (44-215)	7 (3-15)	13 (6-26)	23 (11-43)	33 (16-58)	45 (24-73)
Australia						
All	31 (20-50)	11 (7-17)	20 (13-30)	30 (20-44)	41 (28-57)	52 (37-69)
Females	48 (25-89)	19 (11-33)	33 (19-52)	44 (26-66)	53 (33-76)	64 (42-85)
Males	18 (8-41)	5 (3-12)	11 (5-23)	19 (9-37)	29 (14-53)	38 (19-66)
Brisbane	9 (2-33)	4 (1-15)	9 (3-29)	14 (4-44)	21 (6-57)	28 (9-70)
Sydney	44 (22-88)	14 (7-26)	24 (13-43)	35 (19-58)	47 (27-72)	57 (35-82)
Melbourne	49 (24-98)	15 (8-28)	24 (13-43)	36 (20-59)	47 (27-72)	58 (35-83)

CI = confidence interval

[1]Hazard ratio for melanoma for mutation carriers compared to the general population. P-values for hazard ratio differences between subgroups are: UK all versus Australia all: 0.008; UK females versus UK males: 0.20; Australian females versus Australian males: 0.07; Brisbane versus Sydney versus Melbourne: 0.02

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Table 5.

10-year cumulative risk estimates of melanoma for *CDKN2A* mutation carriers, by region and sex

Geographic region and sex	Cumulative risk of melanoma (%, 95% confidence interval) in the next 10-years for *CDKN2A* mutation carriers who are unaffected at the beginning of the 10-year period			
	For a 40 year-old	For a 50 year-old	For a 60 year-old	For a 70 year-old
United Kingdom				
All	8 (5-14)	12 (7-19)	12 (7-20)	15 (9-26)
Females	10 (4-21)	11 (5-25)	12 (5-25)	13 (6-27)
Males	6 (3-13)	12 (5-24)	13 (6-26)	19 (9-36)
Australia				
All	10 (7-16)	13 (8-19)	16 (10-24)	18 (12-28)
Females	17 (9-29)	16 (9-28)	17 (10-29)	23 (13-38)
Males	6 (3-12)	9 (4-19)	12 (6-25)	13 (6-27)
Brisbane	5 (1-17)	6 (2-20)	7 (2-24)	9 (3-31)
Sydney	12 (6-22)	14 (7-27)	19 (10-34)	19 (10-34)
Melbourne	11 (6-21)	15 (8-27)	18 (9-33)	21 (11-38)