

# The Prevalence of *CDKN2A* Germ-Line Mutations and Relative Risk for Cutaneous Malignant Melanoma: An International Population-Based Study

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## Abstract

Germ-line mutations of *CDKN2A* have been identified as strong risk factors for melanoma in studies of multiple-case families. However, an assessment of their relative risk for melanoma in the general population has been difficult because they occur infrequently. We addressed this issue using a novel population-based case-control study design in which "cases" have incident second- or higher-order melanomas [multiple primary melanoma (MPM)] and "controls" have incident first primary melanoma [single primary melanoma (SPM)]. Participants were ascertained from nine geographic regions in Australia, Canada, Italy, and United States. In the 1,189 MPM cases and 2,424 SPM controls who

were eligible and available for analysis, the relative risk of a subsequent melanoma among patients with functional mutations who have an existing diagnosis of melanoma, after adjustments for age, sex, center, and known phenotypic risk factors, is estimated to be 4.3 (95% confidence interval, 2.3-7.7). The odds ratio varied significantly depending on the type of mutation involved. The results suggest that the relative risk of mutation carriers in the population may be lower than currently believed and that different mutations on the *CDKN2A* gene may confer substantially different risks of melanoma. (Cancer Epidemiol Biomarkers Prev 2006;15(8):1520-5)

## Introduction

Cutaneous malignant melanoma is relatively uncommon (1). Epidemiologic investigations have shown consistently that risk of melanoma is related to phenotypic characteristics such as skin, hair and eye color, and sensitivity to the sun (2). It is also strongly associated with the presence of multiple nevi (3) and dysplastic or atypical nevi (4). However, these observable factors do not fully explain the strong familial aggregation that is frequently observed (5-7). The major gene known to be associated with the development of melanoma, identified from linkage analysis of multiple-case families, is *CDKN2A*, the inhibitor of cyclin-dependent kinase 4 (8, 9). This gene encodes two proteins, p16<sup>INK4A</sup> and p14<sup>ARF</sup>. Germ-line mutations in *CDKN2A* have been found in ~20% of tested multiple-case melanoma families (10, 11). However, they are believed to be rare in the population.

Population-based epidemiologic investigation of rare genetic risk factors is important because evidence suggests that the risk profile may be very different depending on whether the

study is based on multiple-case families or is population based. Indeed, research on *BRCA1* and *BRCA2* mutations in breast cancer shows such a pattern, with higher risks being estimated from studies of multiple-case families and considerably lower risks in population-based investigations (12). Population-based studies of rare germ-line mutations are challenging, however, because they need very large numbers of subjects.

Our goal in this study was to estimate the relative risk of melanoma conferred by *CDKN2A* mutations in the general population. We approached the problem by adopting a novel case-control study design that makes use of the higher frequency of risk factors in patients who have had multiple primary melanomas to increase the statistical power of the study, allowing reasonably precise estimation of the relative risk with feasible sample sizes (13). Controls were participants with an incident first primary melanoma and cases were participants with an incident second- or higher-order melanoma. We have shown that, under certain assumptions, this design gives the same relative risks as a classic case-control study using participants with a single primary cancer and unaffected controls (13). The key assumptions are as follows. First, the two primary melanomas that define a "case" in our study design are independent (i.e., the subsequent primary is not a metastasis of the initial primary). The second assumption is that there is no survival bias (i.e., mutation status does not affect survival following melanoma diagnosis). Finally, we must assume that the relative risk in the high risk segment of the population under investigation, survivors of a first primary melanoma, can be extrapolated to the general population. Our strategy involved sequencing to identify all sequence variants in the region of *CDKN2A* that encodes p16<sup>INK4A</sup>.

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## Subjects and Methods

**Populations.** Participants were recruited to an international multicenter, population-based case-control study of melanoma, the Genes Environment and Melanoma (GEM) Study. The GEM study population consists of incident cases of melanoma identified in eight population-based cancer registries and one hospital center that sees ~50% of the melanoma diagnosed in the state of Michigan. This sample includes nine geographic regions of the world: New South Wales (Australia); Tasmania (Australia); British Columbia (Canada); Ontario (Canada); Turin (Piemonte, Italy); California (Orange County and San Diego County); Michigan (United States); New Jersey (United States); and North Carolina (United States). The study was coordinated at the Memorial Sloan-Kettering Cancer Center. The study design and details of data collection are provided in previous publications (14-16). Briefly, single primary melanoma (SPM) controls were people diagnosed with an incident first invasive primary melanoma in a defined accrual period of 6 months during the year 2000 (the accrual period differed slightly in some centers), and multiple primary melanoma (MPM) cases were individuals diagnosed with an incident second- or higher-order invasive or *in situ* melanoma during a 3.5-year period from January 1 2000 (16). Inclusion of *in situ* cases was designed to avoid exclusion of subjects who could have been diagnosed with an invasive subsequent primary if the *in situ* lesion had not been removed. Physician approval was obtained before subject contact. A 1-hour telephone interview was conducted in which detailed information was obtained on risk factors for melanoma. A short self-administered questionnaire elicited additional risk factor information. Characteristics of melanomas, including anatomic site, Breslow thickness, Clark level, and presence of ulceration, mitoses, and solar elastosis, were obtained from pathology reports. The study protocol was approved by the Institutional Review Board at Memorial Sloan-Kettering Cancer Center and by the Institutional Review Board of each participating institution. All participating subjects signed informed consent.

**Laboratory Methods.** DNA was collected from subjects by means of buccal swabs (88.5%), which were mailed to the participants, or from blood samples (11.5%). Our methods for identifying sequence variation have been described in detail in a previous article (14). Briefly, PCR products spanning exons 1 $\alpha$ , 2, and 3 plus the adjacent intronic regions of all samples were initially screened by denaturing high-performance liquid chromatography analysis (17). All samples showing an altered denaturing high-performance liquid chromatography chromatographic profile were reamplified from genomic DNA in an independent PCR reaction for sequencing. Sequencing was accomplished for exons 1 $\alpha$ , 2, and 3 of the *CDKN2A* gene, the exons that code for the p16<sup>INK4A</sup> protein. We did not sequence exon 1 $\beta$ , which, together with exons 2 and 3, codes for the p14<sup>ARF</sup> protein. Mutations were considered "functional" if they were in the coding region and changed the amino acid sequence or if they were in a noncoding region and known to inhibit transcription of wild-type p16 based on previous *in vitro* studies (18). Common polymorphic variants reported in the literature at codon 148 in exon 2 (*Arg148Thr*) and at nucleotides 500 and 540 in the 3' untranslated region were not considered to be functional variants. The primers extended to regions on either end of the coding regions. However, the primers did not extend to the deep intronic mutation *IVS2-105*, which has recently been associated with melanoma in some melanoma-prone families (19).

**Statistical Analysis.** We compared characteristics of carriers and noncarriers by use of stratified versions of the Kruskal-Wallis test, the Cochran-Mantel-Haenszel test, or the Wilcoxon-Mann-Whitney test, stratified by MPM case versus SPM control status, depending on whether the characteristic was

continuous, binary, or ordinal, respectively. The comparisons of MPM cases with SPM controls were made by conventional methods for case-control studies. Adjusted odds ratio estimates were obtained using logistic regression, controlling for age, sex, geographic region, and relevant phenotypic characteristics: mole count on back, categorized as <5, 5-10, 11-25, and >25; hair color, categorized as dark brown or black, light brown or blonde, and red; eye color categorized as dark (brown) or light (blue, gray, green, hazel); freckles in childhood (none, few, many); propensity to tan (yes/no); and propensity to burn (yes/no).

Our cases are incident cases of second- or higher-order melanoma, and thus the ideal control group would be melanoma survivors who were alive at the time the cases were identified. Because this would be a difficult population to ascertain, we elected to use incident cases of first primary melanoma as controls. We view this as an advantage as it allows cases and controls to be ascertained in exactly the same manner, via population-based registries, and it also allows us to benchmark the frequencies of risk factors in our control group against the cases in conventional case-control studies. The disadvantage is that the design is susceptible to survival bias. However, any risk factor that affects survival will be associated with the times from the dates of diagnosis of the index diagnoses in cases to the dates of the prior diagnoses, and so we were able to test if there was any bias in survival with respect to *CDKN2A* status by comparing, among MPM cases who were second primaries, the times from the first primary melanoma diagnosis to the second primary melanoma diagnosis between those cases who were *CDKN2A* carriers and those who were not. This approach is analogous to a strategy for evaluating survival bias in conventional case-control studies that use prevalent cases (20). We also conducted sensitivity analyses with and without *in situ* MPM cases to determine if the results were materially affected by their inclusion.

The fact that an appreciable number of SPM controls became eligible to be and were ascertained as MPM cases during the accrual period is a further unusual feature of this study. Epidemiologic theory indicates that it is appropriate to retain controls who crossover in this manner as both cases and controls, but this is rarely done in conventional case-control studies because the problem is insignificant (21). In this study, however, the number of crossovers was relatively large ( $n = 96$ ). We have conducted analyses with these case and control patients included and excluded to assess the effect on the results.

## Results

Study recruitment is detailed in Table 1. The participation rates (i.e., the proportions of eligible subjects who completed the questionnaire and provided DNA specimens) were 54% for SPM controls and 52% for MPM cases. An additional 67 (1.9%)

**Table 1. Recruitment of participants**

	SPM, N (%)	MPM, N (%)
Eligible and approached*	4,560 (100)	2,308 (100)
Nonparticipants	2,090 (46)	1,098 (48)
Deceased before contact	170 (3.7)	94 (4.1)
Physician refusal	172 (3.7)	92 (3.9)
Patient refusal	1,330 (29.2)	644 (27.9)
Lost to follow-up	418 (20.0)	268 (11.6)
Participated	2,470 (54)	1,210 (52)
Successful DNA amplification	2,424 (98.1)	1,189 (98.3)

\*Ninety-six patients are in both the MPM and the SPM groups. Ninety-three of the 96 patients had successful DNA amplification. Thus, the total number of individual participants available for genetic analysis is 3,520.

**Table 2. Characteristics of subjects with functional CDKN2A mutations**

Characteristic	SPM		MPM		P*
	CDKN2A-, N = 2,394	CDKN2A+, N = 30	CDKN2A-, N = 1,154	CDKN2A+, N = 35	
Mean age (y)	55	47	65	53	<0.001 <sup>†</sup>
Male (%)	51	43	66	51	0.06 <sup>‡</sup>
Dark eyes (%)	20	26	17	14	0.72 <sup>‡</sup>
Dark hair (%)	32	38	26	17	0.74 <sup>‡</sup>
High propensity to burn (%)	44	30	49	59	0.85 <sup>‡</sup>
High propensity to tan (%)	59	80	53	60	0.03 <sup>‡</sup>
Lack of freckles (%)	45	52	41	34	0.94 <sup>‡</sup>
>10 nevi (%)	42	48	45	61	0.07 <sup>‡</sup>
Family history of melanoma (%)	12	27	21	51	<0.001 <sup>‡</sup>
<i>In situ</i> (%)	0	0	38	31	
Breslow thickness (%)					
0.01-1.00	69	78	75	87	0.07 <sup>§</sup>
1.01-2.00	19	14	15	13	
2.01-4.00	8	4	7	0	
>4.00	4	4	3	0	

\*P value for difference between CDKN2A+ and CDKN2A- adjusting for whether MPM or SPM.

<sup>†</sup>Kruskal-Wallis test (ANOVA).

<sup>‡</sup>Cochran-Mantel-Haenszel test.

<sup>§</sup>Wilcoxon-Mann-Whitney test.

were excluded due to failure to successfully amplify the DNA. Participants were generally representative of the populations from which they were drawn: women were a little more likely to participate than men (56% versus 51%); mean ages were similar in both groups (58 in participants versus 60 in nonparticipants); median Breslow thickness was similar (0.56 versus 0.55); and the distribution of anatomic sites was essentially identical.

Thirty-two different mutations were observed and deemed to be functional in 63 participants. Details of the mutations are catalogued in an earlier report (14). They include 4 insertions or deletions affecting p16<sup>INK4A</sup> in 11 participants; 16 missense mutations affecting p16<sup>INK4A</sup> in 22 participants; 9 missense mutations affecting both p16<sup>INK4A</sup> and p14<sup>ARF</sup> in 17 participants; 2 missense mutations affecting p14<sup>ARF</sup> alone in 4 participants; and 1 mutation upstream of the coding region, but known to affect transcription, in 9 participants (-34G>T). These mutations occurred in 30 of the 2,424 (1.2%) SPM controls and 35 of the 1,189 (2.9%) MPM cases; two carriers were eligible for and are included in both MPM case and SPM control groups. Mutation carriers were relatively evenly distributed among the centers: Vancouver, 3 (7.3%) MPM and 3 (2.5%) SPM; California, 4 (4.6%) MPM and 6 (2.8%) SPM; Michigan, 2 (2.9%) MPM and 5 (1.6%) SPM; New Jersey, 3 (1.9%) MPM and 1 (0.6%) SPM; New South Wales, 12 (2.0%) MPM and 7 (1.0%) SPM; North Carolina, 1 (3.5%) MPM and 2 (0.7%) SPM; Ontario, 5 (3.7%) MPM and 5 (1.2%) SPM; Tasmania, 4 (6.4%) MPM and 0 (0.0%) SPM; and Italy, 1 (5.9%) MPM and 1 (0.8%) SPM.

The characteristics of the mutation carriers are compared with those of the noncarriers in Table 2. CDKN2A carriers developed melanoma at significantly younger ages than noncarriers and reported significantly more family history of melanoma. Carriers and noncarriers are broadly similar in terms of the "phenotypic" characteristics that are known to be associated with melanoma. However, there were P values close to 0.05 for differences with respect to propensity to tan and nevus count, with both greater in carriers.

The estimate of relative risk for the association of any CDKN2A mutation with subsequent primary melanoma, adjusted for age, sex, center, and the phenotypic risk factors listed in Table 2, was 4.3 (95% CI, 2.3-7.7; Table 3). The relative risk estimate was a little less for models that excluded phenotypic factors from the covariates (Table 3). MPM cases are substantially older than SPM controls, as might be expected based on the fact that they have lived long enough

to develop a subsequent melanoma. However, there is no difference in age at first diagnosis of melanoma between cases and controls in this study.

The individual mutations are grouped by broad categories in Table 4. Whereas the overall numbers are small, the results suggest that different categories of mutation confer different relative risks (P = 0.05), with missense mutations that affect both p16<sup>INK4A</sup> and p14<sup>ARF</sup> [odds ratio, 8.7; 95% confidence interval (95% CI), 2.9-26.5] and the noncoding mutation at -34G>T (odds ratio, 15.3; 95% CI, 2.9-79.6) conferring the greatest increases in risk.

We did several additional analyses to assess the sensitivity of the relative risk estimate to choices made in the design of the study. To evaluate survival bias, we measured the times from first to second diagnosis of melanomas in MPM cases with second primaries and compared them in participants with CDKN2A mutations to those without CDKN2A mutations (means, 5.1 versus 5.0 years; P = 0.95). The difference is very small and not statistically significant, indicating that our relative risk estimates are not biased by any effect of CDKN2A mutation on survival from melanoma. When SPM controls who subsequently became MPM cases were excluded from the SPM group, the relative risk adjusted for all factors increased from 4.3 to 4.6 (95% CI, 2.5-8.2). We also repeated all analyses after excluding patients who qualified as MPM cases by virtue of an *in situ* lesion. The mutation frequency in MPM cases increased from 2.9% to 3.3%, and the relative risk estimate after adjusting for age, sex, center and phenotypic factors increased correspondingly from 4.3 to 5.3 (95% CI, 2.9-9.9).

**Table 3. Relative risk of a functionally-relevant CDKN2A variant**

	Relative risk*
Unadjusted	2.4 (1.5-4.0)
Adjusted for age	3.8 (2.3-6.3)
Adjusted for age, center	3.9 (2.3-6.7)
Adjusted for age, center, sex	4.0 (2.3-6.8)
Adjusted for age, center, sex, and phenotypic factors <sup>†</sup>	4.3 (2.4-7.7)

\*Analyses are based on 35 mutations in 1,189 MPM cases and 30 mutations in 2,424 SPM cases.

<sup>†</sup>Nevus count on back, hair color, eye color, freckles in childhood, propensity to tan, and propensity to burn; all of these are statistically significant predictors of risk in this study except eye color.

**Table 4. Cases and controls by individual mutations**

Type of mutation	SPM	MPM	Odds ratio* (95% CI)
No functional mutation	2,394	1,154	1.0
Insertion or deletion <sup>†</sup>	8	3	0.8 (0.2-3.3)
Missense (p16 only) <sup>‡</sup>	12 <sup>§</sup>	11 <sup>§</sup>	3.9 (1.6-9.3)
Missense (p16 and p14) <sup>  </sup>	5 <sup>¶</sup>	13 <sup>¶</sup>	8.7 (2.9-26.5)
Missense (p14 only) <sup>**</sup>	3	1	0.7 (0.1-6.8)
Noncoding transcriptional change <sup>††</sup>	2	7	15.3 (2.9-79.6)

\*Adjusted for age, sex, center, and age-sex interaction.

<sup>†</sup>Mutations observed: 32\_33 ins (24 bp; n = 6), 8\_33 del (24 bp; n = 2), 87\_89 delG (n = 2), 131\_132 insA (n = 1).

<sup>‡</sup>Mutations observed: Leu16Arg (n = 2), Gly23Arg (n = 1), Gly23Ser (n = 1), Arg24Pro (n = 2), Leu32Pro (n = 2), Pro41Pro+Ile49Thr (n = 1), Tyr44Stop (n = 1), Ile49Thr (n = 1), Ile49Ser (n = 1), Gln50Pro (n = 1), Ala57Val (n = 2), Arg58Gln (n = 1), Ala60Val (n = 1), His83Gln (n = 1), Arg124Cis (n = 1), Asp125His (n = 3).

<sup>§</sup>One patient with mutation Gln50Pro is both a case and a control.

<sup>||</sup>Mutations observed: Met53Ile [c.159G>A (n = 2) and c.159G>C (n = 2)], His83Tyr (n = 1), Arg99Trp (n = 1), Gly101Trp (n = 6), Ala102Thr (n = 1), Ala102Ala+Asp108Asn (n = 1), Asp108Asn (n = 1), Arg112Gly (n = 2). Readers may notice that the results differ by two from an earlier report that we published using these data (14). Additional pathology review led us to exclude some participants due to reclassification of prior invasive tumors as *in situ*, and two of these exclusions were mutation carriers, one at Met53Ile and one at Ser56Ile.

<sup>¶</sup>One patient with mutation Met53Ile is both a case and a control.

<sup>\*\*</sup>Mutations observed: Arg58Arg (n = 1); Val106Val+Ala148Thr (n = 3).

<sup>††</sup>Mutation observed: -34G>T (n = 9).

## Discussion

Our study was designed to estimate the relative risk of melanoma in *CDKN2A* carriers in a population-based fashion. Although the study design is unusual, comparing cases with MPM to controls with SPM, it is population based in the sense that both the case and control groups represent population-based ascertainment of incident cases of melanoma. The key question is whether the relative risk estimate we observed (4.3) can be reasonably extrapolated to the related population of real interest, the entire population from which the cases and controls arose.

Previous studies have not been constructed to estimate the relative risk due to a *CDKN2A* mutation, but absolute risk in carriers has been estimated, most prominently in the study of melanoma-prone families reported by the Melanoma Genetics Consortium (22). In this study, the authors reported results from 80 carrier families worldwide with multiple occurrences of melanoma in the families and estimated an average lifetime risk to age 80 of 67% (22). In the light of the fact that the lifetime population risk of melanoma is in the range of 2% to 3%, our relative risk estimate of 4.3 seems to be lower than we might have expected. Consequently, we need to consider carefully the assumptions underlying our study design to assess whether it is reasonable to extrapolate the estimate of 4.3 to the general population.

The three key assumptions are, first, that the different tumors identified in our MPM "cases" are genuinely biologically independent; second, that there exists no survival bias; and third, that the relative risk can be assumed to be constant regardless of the underlying risk in the subpopulation examined—in our case, patients with an existing diagnosis of melanoma.

The first assumption of our novel design (see Introduction), that the case-defining lesion that qualifies a case as MPM is an independent occurrence of melanoma and not a "clone" of the original primary, is supported by several lines of evidence. The degree of clonality of "independent" primary cancers has been examined in numerous studies in recent years using mutation profiling of genes that occur frequently in tumors, such as *p53*, or loss of heterozygosity of selected markers. If the patterns of the mutations in the tumors are similar, then they

are considered to be clonal. In general, these studies have shown that clonality is quite common for mucosal cancers of the head and neck (23) and bladder (24). However, for sites with paired organs, such as breast (25-28) and lung (29-31), the vast majority of new contralateral primaries seem to be biologically independent. The issue does not seem to have been studied in melanoma, but the wide anatomic distribution of melanomas and the absence of a plausible mechanism for the seeding of clonal cells in distant parts of the skin argue against the frequent clonality of multiple primaries in this disease. Importantly, the anatomic site of the two melanomas in individuals with multiple primaries is poorly concordant ( $\kappa = 0.14$ ), with 334 (28%) of the cases having the two lesions in the same general anatomic region, versus 190 (16%) expected (16). Moreover, the weak clustering by site that was observed could be due to variation in amount and pattern of sun exposure by site. Thus, whereas it is certainly possible that some of our "cases" were clonal products of the first primary, we believe that, at worst, this was a rare event, and thus unlikely to have had a substantial influence on the relative risk estimate.

The second assumption is that there is no survival bias caused by differential survival of patients with *CDKN2A*-associated tumors versus those without mutations. However, we found no evidence of this in our comparison of times between index tumors in our GEM cases.

The third assumption is that the relative risk can be assumed to be constant across risk groups. Here the evidence is much less clear-cut. The base population for the GEM study is survivors of an existing melanoma, a group that is necessarily at higher baseline risk than the general population from which these patients with melanoma arose. In previously reported analyses of GEM data for known phenotypic risk factors, our analyses showed statistically significantly elevated risks for mole count, freckling in childhood, propensity to tan, hair color, and family history of melanoma; however, in all cases, the relative risk estimates are lower than summary estimates from available meta-analyses (16). A further related analysis of the GEM study involved examining the incidence of melanoma in first-degree relatives of GEM participants (14). In this study, we observed lifetime risk estimates of 19% for mutations found in SPM controls and 35% for those found in MPM cases. Comparison of the melanoma incidence rate in first-degree relatives of carrier probands versus first-degree relatives of noncarrier probands, using a Poisson regression analysis adjusted for age, sex, geographic site, and case-control status of the proband, leads to a rate ratio estimate of 4.5, which corresponds to a rate ratio of 8.1 (95% CI, 4.7-13.4) in *CDKN2A* carriers versus noncarriers, somewhat higher than the relative risk estimate in our case-control comparison. (The adjustment from 4.5 to 8.1 accounts for the fact that only approximately one half of the first-degree relatives of proband carriers are themselves carriers.) All these results are consistent with the notion that relative risks may be attenuated in higher-risk populations such as people who have had a previous melanoma. The first-degree relatives of GEM participants are a lower-risk population because they have not necessarily had a melanoma diagnosis. It is thus plausible that the observed relative risk estimate of 4.3 in the present study is attenuated simply because it is estimated in a population at high baseline risk of melanoma.

Our results also suggest that the relative risks may vary among mutation carriers depending on the variant. Fifty-one of the 80 (64%) multiple-case families in the Melanoma Genetics Consortium study had missense mutations in the reading frames for both p16<sup>INK4A</sup> and p14<sup>ARF</sup>, whereas this is the case for only 27% (17 of 63) of the carriers identified in our study ( $P < 0.001$ ). (If we had genotyped exon 1 $\beta$  in our study, the percentage of mutations in the common reading frame would have been even lower than the reported 27%.)

The overrepresentation of mutations in this region of the gene in multiple-case families suggests that they confer a higher risk than mutations in other regions. This hypothesis is supported by the relative frequencies in Table 4, which suggest an elevated MPM (case) to SPM (control) ratio for mutations in the joint reading frame relative to the preponderance of the other mutation categories. On the other hand, the noncoding mutation at  $-34G>T$ , heavily represented among GEM cases, occurred in only one of the Melanoma Genetics Consortium families. Overall, the data from Table 4 provide tantalizing evidence that the risks conferred by different mutations vary widely.

We found a *CDKN2A* mutation frequency of 1.2% in incident cases of first primary melanoma, which is considerably higher than the 0.2% estimated in the one previous population-based study (32). This difference is probably explained by the fact that we undertook direct sequencing to identify all possible mutations in the coding region after denaturing high-performance liquid chromatography screening of all participants. The previous study examined only six relatively common candidate variants among their 214 medium risk cases, 201 low risk cases, and 200 control subjects, sequencing only the high-risk cases. In our large population-based analysis, we have found that mutations occur in many locations along the gene. Therefore, sequencing is essential for studies of prevalence and relative risk of *CDKN2A* in the general population.

In summary, our results indicate a much higher prevalence of *CDKN2A* mutations in people with newly diagnosed melanoma than had previously been published. Correspondingly, we have estimated the relative risk of melanoma associated with such mutations to be high (relative risk, 4.3) but considerably lower than previous evidence would suggest. Our data also provide suggestive evidence that there is considerable variation in risks conferred by different mutations and that a population-based approach will identify the lower-risk variants with greater frequency than studies of multiple-case families. We also found no strong associations between carrier status and any of the known phenotypic risk factors for melanoma. These results suggest that *CDKN2A* mutations exert their effect on risk essentially independently of other known risk factors (see also ref. 33).

## Appendix A

The study was conducted by the GEM Study Group: Coordinating Center, Memorial Sloan-Kettering Cancer Center, New York, NY: Marianne Berwick (Principal Investigator, now at the University of New Mexico), Colin Begg (Co-Principal Investigator), Irene Orlow (Co-Investigator), Urvi Mujumdar (Project Coordinator), Amanda Hummer (Biostatistician), Klaus Busam (Dermatopathologist), Pampa Roy (Laboratory Technician), Rebecca Canchola (Laboratory Technician), Brian Clas (Laboratory Technician), Javier Cotignola (Laboratory Technician), Yvette Monroe (Interviewer).

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