BRIEF REPORT | Genetics inMedicine

Phenocopies in melanoma-prone families with germ-line *CDKN2A* mutations

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Purpose: Carriers of *CDKN2A* mutations have high risks of melanoma and certain other cancers. In this study we examined the occurrence of tumors among *CDKN2A* wild type (wt) members of melanoma-prone families with *CDKN2A* mutations.

Methods: Swedish and US melanoma-prone families with *CDKN2A* mutations were included. Data was collected on tumors diagnosed among family members. Among the *CDKN2A* mutated families, members with *CDKN2A* wt status who were diagnosed with melanoma were designated phenocopies.

Results: Of patients with melanoma in the *CDKN2A* mutated families ($n = 266$), 7.1%, were seen among members with *CDKN2A* wt status (phenocopy rate). Among the *CDKN2A* wt family members of the *CDKN2A* mutated families ($n = 256$), 7.4% were diagnosed with melanoma. The prospective relative risk for melanomas was significantly higher among the *CDKN2A* wt subjects compared with population-based controls (7.4 (95% confidence interval 1.7–33.2)), while no elevated risks of non-melanoma cancers were seen and their offspring did not have significantly elevated risks of melanoma or other cancers.

Conclusion: Members of *CDKN2A* mutation carrying families who test negative for their family's mutation have moderately increased risk for melanoma and should, in addition to being considered for continuing dermatologic surveillance, be encouraged to follow sun safety recommendations and practice skin self-exams.

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Key Words: cancer; *CDKN2A* gene mutations; familial melanoma; phenocopies; wild type (wt)

INTRODUCTION

Carriers of pathogenic mutations in the *CDKN2A* tumor suppressor gene have high risks of melanoma and certain other cancers.[1–3] Similar to other cancer susceptibility genes like *BRCA1* and *BRCA2*, some cancer patients in families with a mutation do not carry their family's mutation and are designated phenocopies.[4–7] Phenocopies may result from multiple factors, including risk-modifying genes, environmental effects, ascertainment bias, follow-up, or chance. Until now there has been limited knowledge about phenocopies in *CDKN2A* mutated families. It is thus uncertain what advice noncarriers in *CDKN2A* mutated melanoma families should be given concerning their risk of melanomas or other cancers. In this study we examined the occurrence of cancers among *CDKN2A* wild type (wt) members of Swedish and US melanoma-prone families with *CDKN2A* mutations.

MATERIALS AND METHODS

In Sweden, melanoma-prone families were identified through a national preventive program.[2,8] Family members were invited to undergo germ-line *CDKN2A* mutation analysis for the study. Informed consent was obtained from all participants and the study was approved by local ethical review boards. The national 10-digit personal identity number of *CDKN2A* genotyped family members was linked with the Swedish Cancer Registry, the Multi-Generation Registry, and the Population Registry. In the Cancer Registry, established in 1958, all cancers diagnosed in Sweden are registered (except for basal cell carcinomas).[9] The Multi-Generation Registry contains connections between all individuals born after 1931 and their biological parents.[10] The registry linkage enabled the identification of comprehensive data on census and cancer diagnoses among the *CDKN2A* genotyped family members, their offspring, and population-based matched controls (case to control ratio 1:10). Follow-up started on the date when the first family member was identified in each family (same date in family members and corresponding controls). Follow-up ended at the date of death, emigration, or census date of 31 December 2011.

In the United States, melanoma-prone families with *CDKN2A* mutations were part of a non-population-based family study that has been previously described.[1,11] Briefly, participating families were referred by health-care professionals or through self-referrals. Written informed consent was obtained prior to participation under a National Cancer Institute institutional review board–approved protocol. All family members willing to participate were clinically evaluated, including complete skin examination and routine

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Table 1 Frequencies of phenocopies[a] in Swedish and US melanoma families with *CDKN2A* mutations

	Swedish families *n/n* (%)	US families *n/n* (%)	Total *n/n* (%)
No. families with phenocopies/All *CDKN2A* mutated families	7/34 (20.6%)	7/27 (25.9%)	14/61 (22.9%)
No. phenocopies/All melanoma cases in *CDKN2A* mutated families	9/116 (7.8%)	10/150 (6.7%)	19/266 (7.1%)
No. phenocopies/All *CDKN2A* wt family members in *CDKN2A* mutated families	9/128 (7.0%)	10/128 (7.8%)	19/256 (7.4%)

[a]*CDKN2A* wild type (wt) melanoma cases from *CDKN2A* mutated melanoma families.

medical history. Blood was collected primarily for genetic studies. All families were Caucasian and resided in various regions of the United States. The families have been prospectively followed for up to 40 years starting in the 1970s. Follow-up included periodic clinical evaluation and regular requests for updated medical information including the occurrence of cancer. All cancer diagnoses reported were confirmed by review of histologic materials, local pathology reports, medical records, or death certificates. At both sites, mutation testing was performed in available melanoma patients from each family. If a *CDKN2A* mutation was detected, all available first-degree relatives of cases were tested.

Using data from the Swedish registries, prospective relative risks for melanomas, squamous cell skin cancers, and non-skin cancers were calculated from incidence rates (number of cancers/person-years). All statistical tests were two-sided. Statistical analyses were performed with StatSoft Statistica software, version 10 (StatSoft, Palo Alto, CA, USA).

RESULTS

Among *CDKN2A* mutated families, members with *CDKN2A* wt status, diagnosed with melanoma, were defined as phenocopies. In Sweden and the United States, 34 and 27 melanoma families were identified with pathogenic *CDKN2A* mutations, of whom 7 (20.6%) and 7 (25.9%) families, respectively, had phenocopies (**Table 1**). The *CDKN2A* mutations in the families with phenocopies are shown in **Supplementary Table S1** online. In total, 116 and 150 patients with melanoma (invasive and in situ) were documented in the Swedish and US *CDKN2A* mutated families, respectively, of whom 9 (7.8%) and 10 (6.7%), respectively, were seen among members with *CDKN2A* wt status, representing the phenocopy rates in these families. Among *CDKN2A* wt family members within the *CDKN2A* mutated families, melanomas were diagnosed among 7.0% and 7.8% of the family members who participated in the study, respectively, at each site.

The median age at diagnosis of melanoma in the phenocopies was 47 and 46 years in the Swedish and US families, respectively (**Table 2**). The phenocopy melanoma patients were seen at similar frequencies among women and men. A greater percentage of phenocopy melanoma cases were diagnosed before their family was identified at each of the sites. The vast majority of phenocopies were diagnosed with only one primary melanoma; only one case from Sweden (5.3% of all 19 phenocopies at both sites) had multiple primary melanomas. One US phenocopy case died from melanoma while the remaining phenocopy patients at both sites were alive at the end of follow-up. One US phenocopy case was diagnosed with squamous cell skin cancers and two with non-skin cancers (breast and colon cancer). In total, 43 children of phenocopies, with available follow-up data, were identified (median age at end of follow-up was 25, range 3–57 years). Among these children there was one diagnosis of squamous cell skin cancers but no diagnoses of melanomas or non-skin cancers.

Using data from Swedish registries, cancer risks were calculated in *CDKN2A* wt members ($n = 128$) of Swedish *CDKN2A* mutated families (**Supplementary Table S2**). Median age of the *CDKN2A* wt individuals at baseline was 33 years (range 3–79 years). At baseline seven *CDKN2A* wt individuals had been diagnosed with melanoma, which was significantly greater than among age and sex matched population-based controls ($P < 0.001$). In contrast, at baseline, there was no significant increase in the frequencies of other cancers among the *CDKN2A* wt individuals, compared with controls. The median age at end of follow-up was 51 years (range 22–88). During follow-up, three subjects were diagnosed with melanoma among the *CDKN2A* wt individuals, resulting in a significantly increased prospective risk for melanoma compared with controls (7.4 (95% confidence interval 1.7–33.2)). Similar to baseline, there were no significant differences in prospective risks for other cancers in the *CDKN2A* wt individuals compared with controls.

From the Swedish Multi-Generation Registry, 185 children of *CDKN2A* wt family members were identified, of whom 37.3% were born during the follow-up period (**Supplementary Table S2**). Median age of the children at the end of follow-up was 22 (range 1–64). One child of a *CDKN2A* wt subject was diagnosed with a melanoma at baseline, but no melanomas were diagnosed during the follow-up period. Although there was limited power because of the young ages of the children, there were no statistically significant differences in the frequencies or risks of melanomas or other cancers diagnosed at baseline or in the prospective period. Additionally, at the end of follow-up, among 109 grandchildren of *CDKN2A* wt family members, no melanomas or other cancers had been diagnosed (median age 15 years, range 1–42).

DISCUSSION

This study demonstrates that, in two separate follow-up programs for melanoma-prone families with *CDKN2A* mutations, melanomas among *CDKN2A* wt family members were seen at very comparable frequencies (**Table 1**).

Table 2 Characteristics of phenocopies[a] and their children in Swedish and US melanoma families with *CDKN2A* mutations

Phenocopies	Phenocopies from Swedish families $n = 9$	Phenocopies from US families $n = 10$	Total $n = 19$
Age (years) at first melanoma diagnosis, median (range)	47 (18–68)	46 (25–67)	46 (18–68)
Sex,> *n* n(%)n			
Female	5 (55.6%)	4 (40.0%)	9 (47.4%)
Male	4 (44.4%)	6 (60.0%)	10 (52.6%)
Retrospective and prospective melanomas, *n* (%)			
Melanoma before start of follow-up	7 (77.8%)	7 (70.0%)	14 (70%)
Melanoma after start of follow-up	3 (33.3%)	3 (30.0%)	6 (30%)
Melanoma and cancer diagnoses, *n* (%)[b]			
Multiple primary melanoma	1 (11.1%)	0 (0.0%)	1 (5.3%)
Squamous cell skin cancer	0 (0.0%)	1 (10%)	1 (5.3%)
Non-skin cancer	0 (0.0%)	2 (20%)	2 (10.5%)
	Children of phenocopies from Swedish families	**Children of phenocopies from US families**	**Total**
Children of phenocopies	*n* = 28	*n* = 15[c]	*n* = 43
Age (years) at census date, median (range)	20 (3–57)	35 (5–51)	25 (3–57)
Melanoma and cancer diagnoses, *n* (%)[b]			
Melanoma	0 (0.0%)	0 (0.0%)	0 (0.0%)
Squamous cell skin cancer	0 (0.0%)	1 (6.7%)	1 (2.3%)
Non-skin cancer	0 (0.0%)	0 (0.0%)	0 (0.0%)

[a]*CDKN2A* wild type melanoma cases from *CDKN2A* mutated melanoma families. [b]The US phenocopy cases and their children each had one member diagnosed with basal cell carcinoma (BCC). BCCs were not accounted for in the Swedish families since BCCs are not registered in the Swedish Cancer Registry. [c]Among a total of 22 children, follow-up data were available for 15.

Interestingly, the phenocopy melanoma cases were diagnosed at young median ages (47 and 46 years), compared with melanoma cases in the Swedish and US general population (median age > 60 years),[12,13] but approximately 10 years later than among the melanoma cases with *CDKN2A* mutations from these two sets of families.[1,2,14] The frequency of multiple primary melanomas in the phenocopies was also much lower (5.3%) compared with what is seen among *CDKN2A* mutation carriers (~40%).[14,15] This observation does not appear to have been biased by survival differences since all but one of the phenocopy melanoma cases were still alive at the end of follow-up.

Most melanomas among the phenocopies had been diagnosed prior to the identification of their respective family at each site. Nonetheless, in Sweden, the prospective risk for melanomas was also significantly higher among the *CDKN2A* wt subjects compared with population-based controls, although the risk increase was much lower than what is seen among *CDKN2A* mutation carriers.[2] These results suggest that *CDKN2A* wt relatives of *CDKN2A* mutation carriers have melanoma risk and age at diagnosis that is intermediate between population-based cases and *CDKN2A* mutation carriers. This observation supports the premise that other factors, in particular risk-modifying genes and ultraviolet exposure patterns, affect the melanoma penetrance of *CDKN2A* mutation carriers along with the melanoma risk among the *CDKN2A* wt family members.[11,16,17] Consistent with this hypothesis, in a study of 815 *CDKN2A* mutation carriers from centers across Europe, North America, and Australia, variants in the *MC1R* gene were significantly associated with melanoma risk in carriers across all three continents.[17] *CDKN2A* mutation carriers with certain single *MC1R* variants had significantly elevated odd ratios for melanoma (odds ratio up to 4.7) and two or more variants had odds ratios even higher. Hence, it is likely that many *CDKN2A* families that are identified for their melanoma propensity also have melanoma-associated variants in *MC1R* and likely other low-risk melanoma susceptibility genes such as *TYR*, *TYRP*, and *ASIP*. In this study, we did not add data on any low-risk melanoma susceptibility gene variants, but it is likely that such variants play a role in the occurrence of melanoma among noncarriers of their family's *CDKN2A* mutation. Several additional high-risk melanoma susceptibility genes have been identified, including *CDK4*, *POT1*, *BAP1*, *TERF2IP*, and *TERT* promoter.[18] Since mutations in *CDKN2A* and these high-risk melanoma genes are extremely rare in the population it is very unlikely that such mutations would co-occur in the same family. Hence, although the families in this study have not been screened for these genes, it is unlikely that such high-risk mutations would be involved in the melanoma risk for phenocopies in the *CDKN2A* mutated families.

Further, it is possible that the ascertainment of melanoma families and dermatologic follow-up results in increased rates of melanoma phenocopies; however, despite differing programs for inclusion and follow-up in Sweden and the United States, very similar phenocopy rates were seen at both sites. In contrast to what has been observed and reported among *CDKN2A* mutation carriers,[2,3,19,20] the *CDKN2A* wt family members in this study did not appear to have elevated risks of nonmelanoma cancers. In addition, the offspring of the *CDKN2A* wt members did not show any evidence for increased risks of melanoma and other cancers. Of note, basal cell carcinomas were not included in the risk analyses since these tumors are not registered in the Swedish Cancer Registry. In this study, the presence of phenocopies was studied among 61 families with *CDKN2A* mutations, but similar studies involving other cohorts of *CDKN2A* mutated families are needed to confirm our findings.

When offering genetic testing to families it is important to also have knowledge of the outcomes of individuals who test negative for their family's mutation and to our knowledge this study is the first to address this problem in *CDKN2A* mutated families. The findings from this study may therefore be helpful for clinicians and genetic counselors who encounter families that carry *CDKN2A* mutations. To conclude, members of *CDKN2A* mutation carrying families who test negative for their family's *CDKN2A* mutation remain at moderately increased risk for melanoma (but not for other cancers) and should therefore, in addition to being considered for continuing dermatologic surveillance, be encouraged to follow sun safety recommendations and practice skin self-exams.

SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at http://www.nature.com/gim

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DISCLOSURE

The authors declare no conflict of interest.

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