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Population-Based Prevalence of *CDKN2A* Mutations in Utah Melanoma Families

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Cyclin-dependent kinase inhibitor 2A (*CDKN2A* or *p16*) is the major melanoma predisposition gene. In order to evaluate the candidacy for genetic testing of *CDKN2A* mutations among melanoma prone families, it is important to identify characteristics that predict a high likelihood of carrying a *CDKN2A* mutation. We primarily used a unique Utah genealogical resource to identify independent melanoma prone families whom we tested for mutations in *CDKN2A*, *cyclin-dependent kinase 4*, and *alternate reading frame*. We sampled 60 families which met the inclusion criteria of two or more affected first-degree relatives. We found four different pathogenic *CDKN2A* mutations in five families, mutations of uncertain significance in two families, and known polymorphisms in three families. One of the mutations of uncertain significance, 5′ untranslated region −25C>T, has not been previously described. Among our population-based set of Utah families, the prevalence of *CDKN2A* mutations was 8.2% (4/49); the overall prevalence when physician-referred pedigrees were also considered was between 8.3% (5/60) and 10% (6/60). Having four or more first- or second-degree relatives with melanoma, or a family member with ⩾3 primary melanomas, correlated strongly with carrying a *CDKN2A* mutation. We observed a significantly elevated rate of pancreatic cancer in one of four families with a deleterious *CDKN2A* mutation.

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INTRODUCTION

Melanoma is a devastating malignancy and the most lethal of all skin cancers. Among the top 10 most common cancers in the United States (US) between 1992 and 2001, melanoma had the highest rate of increase in incidence in men and the second highest in women (Jemal *et al.*, 2004). *Familial melanoma* refers to the clustering of several cases within a single family and accounts for about 5–12% of melanoma cases (Goldstein and Tucker, 2001). Being a member of a melanoma-prone family has been associated with a 35–70-fold increase in the relative risk of developing a melanoma (Kefford *et al.*, 1999; Piepkorn, 2000). Three germline melanoma predisposition gene products have been identified, *cyclin-dependent kinase inhibitor 2A* (*CDKN2A* or *p16*) (Cannon-Albright *et al.*, 1994; Hussussian *et al.*, 1994; Kamb *et al.*, 1994), *cyclin-dependent kinase 4* (*CDK4*) (Zuo *et al.*, 1996), and *alternate reading frame* (*ARF*) (Randerson-Moor *et al.*, 2001). The *CDKN2A* gene locus encodes both the *p16* and *ARF* transcripts. Mutations that affect p16 function have been reported in 25–40% of melanoma prone families, while mutations in *CDK4* and *ARF* are relatively rare, accounting for <1% of familial melanomas (Piepkorn, 2000; Hayward, 2003). In this article, *CDKN2A* variants that affect p16 will be referred to as ''*CDKN2A* mutations''.

p16 is a key cell cycle regulatory molecule that inhibits CDK4/6-dependent phosphorylation of retinoblastoma protein to block entry into the G1/S phase of the cell cycle (Ruas and Peters, 1998; Hayward, 2003). The functional result of germline *CDK4* mutations is a loss of ability for this protein to be bound and thus inhibited by p16, resulting in dysregulation of the same retinoblastoma cell cycle pathway. Though also coded by the *CDKN2A* gene locus, ARF is a distinct tumor suppressor that functions through the *human double minute 2* pathway regulating the degradation of p53 (Zhang *et al.*, 1998). Mutations in each of these gene products have been found to cosegregate with melanoma in familial melanoma pedigrees (Zuo *et al.*, 1996; Goldstein *et al.*, 2000; Randerson-Moor *et al.*, 2001; Soufir *et al.*, 2004).

Since *CDKN2A* was identified as the major melanoma susceptibility gene, groups from around the world have identified melanoma-prone families and reported the prevalence rate of *CDKN2A* mutations among them. These studies have identified clinical features associated with *CDKN2A* mutation-positive families, including multiple affected relatives, family members with multiple primary

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Abbreviations: ARF, alternate reading frame; CDK4, cyclin-dependent kinase 4; CDKN2A, cyclin-dependent kinase inhibitor 2A; UPDB, Utah Population Database; UTR, untranslated region

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melanomas, and the presence of some nonmelanoma cancers (eg pancreatic cancer) (Vasen *et al*., 2000; Lynch *et al*., 2002; Puig *et al*., 2005). Previous reports of *p16* mutation rates in familial melanomas are summarized in Table 1. Among these studies, the standard for what constitutes ''familial'' predisposition to melanoma has varied significantly, with inclusion criteria ranging from any person with multiple primaries to at least three affected first-degree relatives over two generations. These differences in inclusion parameters can be well understood in the context of the differing incidences of sporadic melanoma among the respective populations (eg the UK has about 10 cases per 100,000 per year, while Australia reports an annual incidence of about 50 cases per 100,000 per year (Maclennen *et al*., 1992). However, the differences in defining ''familial melanoma'' also make the comparison of these data difficult.

In the majority of studies, participants were recruited from melanoma registries created from melanoma clinics. These registries often rely on participant reports of family history, and data are often limited or inaccurate because the participant (1) may be unfamiliar with his own family tree, (2) has limited knowledge of melanomas within his family, or (3) does not know the difference between melanoma and nonmelanoma skin cancers. In addition, although it is relatively straightforward to confirm the reported cases of melanoma within a family, the effort required to establish that all other family members are truly unaffected is cost prohibitive. Furthermore, in registries developed within tertiary referral or academic centers, there is potential for referral bias of individuals with the most dramatic clinical presentations. The clearest approach to avoid this ascertainment and referral bias is to use a population-based recruitment strategy, as was first done by Platz *et al*. (1997). The impact of recall bias can also be lessened by utilizing existing genealogic records.

The Utah Population Database (UPDB) is a research resource at the University of Utah that contains information for over six million unique individuals using genealogy records from the Family History Library, vital records from the Utah State Department of Health, and other statewide data sets, as described in Florell *et al*. (2005). Information from Utah birth certificates has been successfully linked to the original genealogies to extend the data to the most recent birth cohorts; thus, the genealogy records represent pedigrees that may span up to 10 generations. Birth records are also used to create multigenerational Utah families that were not part of the original data set. These genealogical records are linked to the Utah Cancer Registry, a Surveillance, Epidemiology and End Results Cancer Registry, that has recorded all invasive cancers in Utah since 1966. The combination of statewide cancer records with genealogy data in the UPDB provides a way to objectively confirm cancers in family members and to identify the inter-relatedness among cancer

Table 1. Reported *p16* mutation rates

Study location	Author (year)	Families studied	*p16* prevalence	Definition of melanoma-prone family
Poland	Lamperska *et al*. (2002)	16	0 (0%)	One or two MM cases in the family
Utah, USA	Kamb *et al*. (1994)	36	2 (6%)	Either one MM case and two cases of DNS or at least two MM cases per family
NE, Italy	Landi *et al*. (2004)	55	4 (7%)	Two cases of MM in the family
Israel	Yakobson *et al*. (2000)	30	2 (7%)	Two cases or MPM
Sweden	Platz *et al*. (1997)[1]	64	5 (8%)	Two or more cases of MM (first-, second-, or third-degree relatives)
Australia	Holland *et al*. (1999)	131	11 (8.4%)	At least two MM cases
Australia	Aitken *et al*. (1999)	87	9 (10%)	Population-based, high-risk status assigned based on the number of cases, age or melanoma, sex and birth cohorts
Spain	Ruiz *et al*. (1999)	34	6 (17%)	At least one w/ MM and one w/ DNS
Massachusets, USA	Fitzgerald *et al*. (1996)	28	5 (18%)	Two cases of MM (must be first- or second-degree relative)
Australia	Flores *et al*. (1997)	48	10 (21%)	Three cases of MM in two consecutive generations
UK	Harland *et al*. (1997)	27	6 (22%)	Two cases of MM or MPM
Scotland	Mackie *et al*. (1998)	16	6 (38%)	Two cases of MM
Italy	Fargnoli *et al*. (1998)	10	4 (40%)	2 cases of MM in first- or second-degree relatives
France	Soufir *et al*. (1998)	48	21 (44%)	Three cases of MM, or two cases if one before age 50. Also at least one MPM OR relative w/Panc CA, or two affected first-degree relatives
Total		630	91 (14%)	

[1]This was the first population-based assessment of p16 prevalence rate.
MM=melanoma; MPM=multiple primary melanomas; DNS=dysplastic nevus syndrome; Panc CA=pancreatic adenocarcinoma.

cases. By calculating the internal rates of cancer, the UPDB can be used to determine whether a significant excess of cancer exists in a specific family (Cannon Albright *et al*., 2005). The use of the UPDB to identify and characterize high-risk melanoma families limits biases associated with physician referral and patient recall.

RESULTS

Mutation status

Table 2 summarizes data on the *CDKN2A* mutations we found that were deleterious, of uncertain significance, or polymorphisms. Among the 60 families tested, we identified four previously described deleterious *CDKN2A* mutations in five families. In addition to two polymorphisms (A148T, and −33 5′untranslated region (UTR) G>C) and a mutation of uncertain significance (A4T) also previously described, we found one novel mutation in exon 1 at 5′ UTR −25, resulting in a G>C transition in one family that had two members with melanoma. We were only able to recruit and test the two affected individuals, a parent and child. The parent developed two primary melanomas, in the seventh decade of life, and the child developed a melanoma in its 30s. Both were −25 mutation carriers.

We estimate the proportion of familial melanoma (defined as a cluster of two or more first-degree relatives) due to mutations that affect p16 to be between 8.3% (5/60) and 10% (6/60), depending on whether or not the 5′UTR −25 change is determined to be deleterious. The A4T mutation did not cosegregate with disease in our families. We found no mutations in *ARF* or *CDK4* in any of the 60 families.

CDKN2A mutations and the number of affected first- and second-degree relatives

Table 3 summarizes the mutation screening results and clinical characteristics for the 60 families, according to the number of cases of melanoma in first- and second-degree relatives of the proband. We found six families with four or more affected first- or second-degree relatives, 18 families with three affected relatives, and 36 families with two affected first- or second-degree relatives. We did not count known affected members more distant than second-degree relation to the tested individuals because it is unusual in clinical practice for patients to accurately know the melanoma history of family members who are distantly related. Table 3 summarizes the frequency of *CDKN2A* variants by number of affected first- and second-degree relatives in the family. In all, 50% of the families with four or more first- or second-degree relatives affected had a known deleterious *CDKN2A* mutation.

Multiple primary melanomas

We identified nine families with individuals who had multiple primary melanomas. In Table 4, we show the association of the maximum number of primary melanomas

Table 2. *p16* Variants identified in the 60 Utah families

Mutation Found	Location	Effect/First described	Effect[1]	# of Families affected	Co-segregation with disease
5′UTR −34G>T	Exon 1	Deleterious/Liu *et al.* (1999)	New start site	2	Yes
V126D	Exon 2	Deleterious/Hussussian *et al.* (1994)	Missense	1	Yes
G101W	Exon 2	Deleterious/Hussussian *et al.* (1994)	Missense	1	Yes
32ins24	Exon 1	Deleterious/Harland M. *et al.*	Insertion	1	Yes
A148T	Exon 2	Polymorphism/Hussussian *et al.* (1994)	Missense	3	No
5′UTR −33G>C	Exon 1	Polymorphism/Soufir *et al.* (1998)	None known	1	No
A4T	Exon 2	Unknown significance	Unknown	1	No
5′UTR −25C>T	Exon 1	Unknown significance	Unknown	1	Yes

[1]Data on mutational effect obtained through eMelanobase website (Melanoma Genetics Consortium, 2005).

Table 3. Mutational status by number of affected relatives

		Deleterious mutation		Mutation of uncertain significance		Polymorphism	
Number of melanomas in first- and second-degree relatives including proband	Total number of families (% of total)	Total	Percent	Total	Percent	Total	Percent
2	36 (60)	1	3	1	3	2	6
3	18 (30)	1	7	1	7	0	0
⩾4	6 (10)	3	50	0	0	0	0

Table 4. Mutational status by multiple primary melanomas

Number of primary melanomas in affected families	Number of families (% of total)	Deleterious mutation		Mutation of uncertain significance		Polymorphism	
		Total	Percent	Total	Percent	Total	Percent
1	51 (85)	1	2	0	0	2	4
2	6 (10)	1	16	0	0	0	0
⩾3	3 (5)	3	100	0	0	0	0

observed in an individual with the results of the *CDKN2A* mutation testing in their family. Four of the nine families (44%) with multiple primary melanomas had a *CDKN2A* mutation. All three pedigrees with members that had three or more primary melanomas had a mutation in *CDKN2A*. Only one of the six families with a member having two primary melanomas had a mutation in *CDKN2A*. Of interest, this individual reported having three primary melanomas, but we were unable to confirm the third melanoma, resulting in inclusion in the two primary melanomas category. Though our sample size is limited, the strong correlation between individuals with three or more primary melanomas and a *CDKN2A* mutation in the Utah population is clear.

Pancreatic cancer in melanoma families

We found 22 families with at least one pancreatic cancer case. In 18 of these families more pancreatic cancer cases were observed than expected, but in only four of these families was the excess significant ($P<0.05$).

DISCUSSION

The overall rate of deleterious CDKN2A mutations in our familial melanoma pedigrees was significantly less (8.3–10%) than the oft-quoted range of 25–40%. We observed that these mutations (V126D, −34G>T, G101W, and 32ins24) cosegregated in our pedigrees (data not shown). We report one novel mutation, 5′UTR −25C>T, that cosegregated with melanoma within the only family that carried it. We found that the clinical characteristics associated with an elevated risk of carrying a *CDKN2A* mutation in the Utah families are: (1) having ⩾4 first- or second-degree relatives with melanoma and (2) a first- or second- degree relative with ⩾3 primary melanomas. One of the families with a *CDKN2A* mutation had a significant excess of pancreatic cancer.

The three features of the Utah pedigrees studied that need to be considered in exploring why the *CDKN2A* mutation rate was lower than expected are: (1) the high amount of UV exposure in Utah due to the elevation and sunny environment, (2) the inclusion of previously studied high-risk pedigrees, and (3) the largely population-based recruitment. In the Salt Lake City vicinity, where about half the population of Utah lives, the average elevation is 4,330 feet and it ranks in the top quartile among US cities in the number of sunny days per year (with over 120 on average). The increased UV exposure in such an environment may explain the elevated background rate of melanoma in Utah, which may in turn lead to a larger number of first-degree affected individual pairs where the precipitating cause is environmental and not genetic. Large, well-defined *CDKN2A*-mutation-positive pedigrees with thousands of family members have been studied at our institution for the last 15 years. We include these families as single families (see Table S1), rather than breaking them into arbitrarily defined clusters of two or more first-degree cases. This has the effect of reducing the rate of *CDKN2A* mutations observed. In other published studies, it is not uncommon for a majority of the *CDKN2A* carrier families to have the same mutation (Mackie *et al.*, 1998; Borg *et al.*, 2000; Mantelli *et al.*, 2002), which leads to the speculation that these families may represent parts of larger undefined families like ours (‘‘founder effect’’). Among the five *CDKN2A* mutation-positive families in this study, only two had the same mutation, and we confirmed that there was no genetic relationship between these families. The recruitment of our families without the extensive genealogic data available in Utah would have resulted in an artificially elevated rate of *CDKN2A* mutations.

To minimize referral bias, we recruited largely from our population database. We are aware of two other groups that have used population-based recruitment, the first done in Sweden and the other in Australia. In the Swedish study, Platz *et al.* (1997) found the *CDKN2A* mutation rate in familial melanoma to be 7.8%, while a study conducted later by Borg *et al.* (2000) in the Swedish population used both referral- and population-based means for recruitment, and reported a mutation rate of 19%. In Australia, a population-based assessment of the *CDKN2A* mutation rate carried out by Aitken *et al.* (1999) reported a rate of 10.3%, while a previous study that recruited participants through a registry reported a rate of 20.8% (Flores *et al.*, 1997). When Fitzgerald *et al.* (1996) examined their melanoma-prone population in the US, they found the rate of *CDKN2A* mutation carriage to be about 18%. Our Utah population, has low inbreeding and is representative of the general US Caucasian population (Jorde *et al.*, 2000). Four of the 49 (8.2%) families that we recruited from a population-based resource had a deleterious *CDKN2A* mutation. One to two of the 11 (9.1–18.2%) families that were physician referrals had deleterious mutations. Combining the families recruited by population-based assessment with physician referral had little effect on *CDKN2A* rate, increasing the prevalence rate among our families to

8.3–10%. Based on these observations, it is reasonable to consider that the oft-quoted 25–40% rate of *CDKN2A* mutations in melanoma-prone families may overestimate the rate in the general melanoma-prone family population.

Novel *CDKN2A* mutation

We report one novel *CDKN2A* mutation (5′UTR −25C > T), found in one family that had two members with melanoma, both of whom carried the mutation. Previous mutations have been reported in the 5′UTR region of *CDKN2A*, including the −34 G > T mutation, which creates a false start codon and is a known deteterious mutation (Liu *et al.*, 1999), and the −33 mutation, which is considered a polymorphism with no proven adverse clinical effect (Soufir *et al.*, 1998). A mutation in the untranslated region of *CDKN2A* is unlikely to affect the coding sequence to interfere with CKD4 binding. Other functional testing (eg a retinoblastoma phosphorylation assay, or an assay to assess cell cycle exit) may shed light on the effect of this mutation. It is also worth noting that the −25 position is in a 289 bp CpG island (European Molecular Biology Laboratory–European Bioinformatics Institute, European Bioinformatics Institute, 9 June 2005) containing numerous MZF1- and SP1-binding sites (TF Search against TRANSFAC matrix table rev. 3.3) and the G > C mutation enriches and extends the local CpG island. Also, using the interspecies alignment of the UCSC genome browser version 107 (bp 21,964,828–21,964,865), we found that the −24 and −25 basepairs are unanimously conserved across all mammalian species listed (human, chimp, dog, mouse, and rat) as well as the zebrafish. It will be important to evaluate allele-specific expression analysis in the future to determine whether this is a pathogenic mutation.

CDKN2A and pancreatic cancer

Lynch and Fusaro (1991) reported a relationship between the familial atypical mole syndrome and pancreatic cancer in the early 1990s. Vasen *et al.* (2000) published work documenting the strong association between a 19-bp deletion in *CDKN2A* and pancreatic cancer in Dutch families, and several *CDKN2A* prevalence studies have documented the occurrence of pancreatic cancer in melanoma-prone kindreds (Fitzgerald *et al.*, 1996; Soufir *et al.*, 1998; Yakobson *et al.*, 2000; Parker *et al.*, 2003; Landi *et al.*, 2004). Using the genealogic information available to us through the UPDB, ours is the only study that has addressed the issue of whether the rate of pancreatic cancer is significantly elevated in high-risk melanoma families with and without *CDKN2A* mutations. We are aware of some limitations inherent in relying on a population database for this type of study. Cancers occurring out of the state of Utah or prior to 1966 are censored, and those cancer cases which do not link to genealogical records are not analyzed. We expect that there is no bias in such censoring, and that our estimates of increased risk are conservative. We have used the methods presented here in multiple studies of cancer risk in the Utah population (Goldgar *et al.*, 1994; Cannon Albright *et al.*, 2005). Among our 60 pedigrees, four had significantly elevated rates of pancreatic cancer (see families 2, 11, 47, and 58 in Table S1), and one carried a *CDKN2A* mutation.

In the face of the evidence reported by Vasen *et al.* (2000), which clearly showed an excess rate of pancreatic cancer among their melanoma-prone kindreds with the 19-bp deletion (Leiden mutation), it seems reasonable to propose that perhaps certain *CDKN2A* mutations confer greater risk of both melanoma and pancreatic cancer than other mutations. The elevation and sunny environment, which increase UV exposure in our population, may lead to a preferential expression of the melanoma phenotype without a similar environmental factor to increase the rate of pancreatic cancer (Bishop *et al.*, 2002). The importance of establishing the risk of other cancers or diseases associated with a *CDKN2A* mutation is apparent as genetic testing for the *CDKN2A* mutations becomes more readily available. Genetic counselors and other health professionals may be challenged to tailor their teaching and recommendations to the risks associated with specific populations of *CDKN2A* carriers.

MATERIALS AND METHODS

Eligibility criteria

All patient recruitment was performed in accordance with protocols approved by the University of Utah Institutional Review Board, and all clinical investigation has been conducted according to the Declaration of Helsinki Principles. All participants gave their written, informed consent. We defined a melanoma-prone family as having at least two first-degree relatives (a proband and a first-degree relative) affected with invasive melanoma. This reasonably stringent criterion limits recruitment of sporadic melanomas, and can be easily applied in everyday clinical practice. We attempted to recruit one or more melanoma cases from each pedigree for genetic testing, preferentially recruiting the individuals affected at the youngest age to decrease the chances of selecting a sporadic case.

Ascertainment

We used a population-based recruitment strategy to ascertain families, querying the UPDB to identify families with at least two first-degree relatives with melanoma. From over 4,000 cases of invasive cutaneous melanoma for whom we also have genealogy data available, we identified and recruited 49 independent sets of at least two first-degree relatives with melanoma (families). Families were defined as sets of related melanoma cases who had a common ancestor, among whose descendants there was a significant excess of melanoma cases, as determined by internally estimated rates of melanoma. In addition, we accepted seven physician referrals and four self-referrals that met our ascertainment criteria. These physician and self-referrals were not identified in the UPDB as either the diagnoses were too recent and not yet included in the latest version of the UPDB, or a family member was diagnosed outside the state of Utah. Melanoma diagnoses from physician or self-referrals were objectively confirmed through medical records or review of microscopic slides by a dermatopathologist (SRF).

Mutation testing

p16 mutation testing was performed by Myriad Genetics (Salt Lake City) and Yale Diagnostics (New Haven). The entire coding region of exon 1α, exon 2, exon 3, splice junctions, 5′UTR, and a deep

intronic region involved in a common splice mutation (Harland *et al.*, 2001) were sequenced in both the forward and reverse directions. Exon 2 of *CDK4* and exon 1β and the splice junctions of the *ARF* genes were sequenced in the Yale Diagnostics Laboratory, and revealed no additional mutations.

Melanoma confirmation

UPDB permits the identification of melanoma cases in large, extended pedigrees, but this degree of information is not typically available in a clinical practice. Therefore, we designed our study to consider only the number of cases in a pedigree within a first- or second-degree relationship in the evaluation of clinical features. In the case where multiple individuals from a single kindred were tested, we report the number of first- and second-degree relatives from the tested member who had the most affected relatives.

We confirmed all diagnoses of melanoma in the participants and their family members by the Utah Cancer Registry, medical records, or death certificates.

Pancreatic cancer rates

We tested the hypothesis of a significant excess of pancreatic cancer in each family using a previously published method (Cannon-Albright *et al.*, 2005; see Table S1). From the founding individual of each family, we counted the observed number of pancreatic cancer cases among all the descendants, and compared this to the expected number of pancreatic cancer cases among all the descendants. The expected number of pancreatic cases was calculated using age- and sex- and birthplace-(Utah or not Utah) specific rates of pancreatic cancer estimated within the UPDB, applied to all of the descendants in each family. Of the 60 families, 45 had sufficient genealogic information available (at least three generations of individuals in the UPDB) to facilitate these calculations.

CONFLICT OF INTEREST

The authors state no conflict of interest.

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SUPPLEMENTARY MATERIAL

Table S1. Characteristics of the Utah families.

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