

Rarity of CDK4 germline mutations in familial melanoma

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To date, two genes have been implicated in melanoma pathogenesis. The first, *CDKN2A*, is a tumour suppressor gene with germline mutations detected in 20% of melanoma-prone families. The second, *CDK4*, is an oncogene with co-segregating germline mutations detected in only three kindreds worldwide. We examined 16 American melanoma-prone families for mutations in all coding exons of *CDK4* and screened additional members of two previously reported families with the Arg24Cys germline *CDK4* mutation to evaluate the penetrance of the mutation. No new *CDK4* mutations were identified. In the two Arg24Cys families, the penetrance was estimated to be 63%. Overall, 12 out of 12 invasive melanoma patients, none out of one *in situ* melanoma patient, five out of 13 dysplastic naevi patients, two out of 15 unaffected family members, and none out of 10 spouses carried the Arg24Cys mutation. Dysplastic naevi did not strongly co-segregate with the Arg24Cys mutation. Thus the phenotype observed in melanoma-prone *CDK4* families appears to be more complex than just the *CDK4* mutation. Both genetic and environmental factors are likely to contribute to the occurrence of melanoma and dysplastic naevi in these families. In summary, although *CDK4* is a melanoma susceptibility gene, it plays a minor role in hereditary melanoma. © 2002 Lippincott Williams & Wilkins

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Introduction

Cutaneous malignant melanoma (CMM) is a potentially fatal form of skin cancer whose aetiology is heterogeneous and complex. To date, two genes have been implicated in melanoma pathogenesis. The first, *CDKN2A*, encodes a low molecular weight protein, p16, that inhibits the activity of the cyclin D1–cyclin-dependent kinase-4 (CDK4) complex.¹ Thus, *CDKN2A* acts as a tumour suppressor gene that negatively regulates cell growth by arresting cells at G₁ of the cell cycle. Germline mutations have been detected in approximately 20% of melanoma-prone families (with at least three cases) examined in North America, Europe, and Australia.²

The second melanoma susceptibility gene, *CDK4*, acts as an oncogene,³ and co-segregating germline mutations have been detected in only three melanoma-prone families worldwide.^{4,5} The two germline mutations detected in *CDK4* occurred in the same codon. The Arg24Cys germline mutation, identified in two families,⁴ was first described as generating a tumour-specific protein antigen in sporadic melanoma. The mutation produced an altered protein that prevented binding of the CDK4 protein to p16.³ The second germline mutation, Arg24His, also directly involved in inhibiting binding to p16, has been observed in one French family.⁵

The purpose of this study was to examine 16

American melanoma-prone families for mutations in all of the coding exons of the *CDK4* gene, and to screen additional members of the two families (designated R and S) with the Arg24Cys germline mutation,⁶ recently shown to be a founder mutation,⁶ in order to evaluate the penetrance of this mutation.

Materials and methods

The study subjects came from families in which there was a history of invasive melanoma in at least two first-degree relatives. They came from two sources. Most of the families were referred to the Genetic Epidemiology Branch/National Cancer Institute's ongoing family study of CMM by health-care professionals or through self-referrals. Six families were recruited during a follow-up family study associated with a melanoma case-control study conducted at the University of Pennsylvania and the University of California at San Francisco. All the families were Caucasian and resided in various regions of the United States. Written informed consent was obtained prior to participation under Institution Review Board approved protocols. All diagnoses of melanoma were confirmed using histological review of pathological material, pathology reports or death certificates. Criteria for the diagnosis of dysplastic naevi (DN) have been previously described.^{7,8} Briefly, obligatory criteria for the clinical diagnosis of DN were a size of 5 mm or larger and being entirely flat or having a flat component. At least two of the following were also necessary: variable pigmentation, irregular asymmetrical outline, and indistinct borders. The histological criteria are described in detail by Clark and Tucker.⁸ Participants were classified as having DN if they had clinical or histological evidence of DN. Sixteen families with an average of three melanoma cases per family were screened for *CDK4* mutations using single-strand conformation polymorphism analysis. All families had previously tested negative for *CDKN2A* coding region mutations.⁶ In 10 families, we screened one CMM patient and in the other six families we tested at least two affected subjects for *CDK4* mutations. If a genetic alteration was observed, all available family members were tested for the alteration. The seven coding exons from *CDK4* (i.e. exons 2–8) were polymerase chain reaction-amplified using the primers and conditions described by Zuo *et al.*⁴

Results

The 16 melanoma-prone families without *CDKN2A* mutations had an average of three melanoma patients per family (Table 1). Five families had two CMM patients, eight families had three CMM patients, and three families had five CMM patients. No co-segregating *CDK4* mutations were identified in these families.

Seventeen additional family members from the two families carrying the Arg24Cys mutation were screened for this alteration. Table 2 shows the distribution of mutation and non-mutation carriers in the two families, and the disease status for each individual. All 12 invasive melanoma patients, 38.5% of the DN patients, 13.3% of the unaffected family members and no spouses carried the Arg24Cys mutation. Direct assessment of the family members revealed a penetrance of approximately 63% (12 melanoma patients with the mutation divided by 19 subjects with the Arg24Cys mutation), with a 95% confidence interval of 42–85%.

Discussion

Epidemiological studies of melanoma have demonstrated the importance of DN as a major risk factor for both familial and non-familial melanoma. However, previous examinations of melanoma-prone *CDKN2A* families showed that DN do not appear to co-segregate with *CDKN2A* mutations.^{2,9} Although based on small numbers, we observed a similar pattern in the two Arg24Cys families. There was no strong co-segregation between DN and the *CDK4*

Table 1. Families examined for *CDK4* mutations

Family	Confirmed CMM cases per family	CMM subjects tested per family
3340	2	1
3341	3	2
3356	3	1
3364	5	2
3374	3	3
3436	3	1
B1	2	2
B2	2	1
AA	2	1
BB	3	1
DD	2	1
AM	4	2
AN	5	1
AS	3	1
W	3	1
Z	5	2

Table 2. Numbers and percentages of Arg24Cys mutation carriers in the two American melanoma-prone families (families R and S)

Subject type	Mutation carriers		Total subjects		Totals for both families	
	R	S	R	S	Mutation carriers/total subjects	%
Invasive melanoma	7	5	7	5	12/12	100
Melanoma <i>in situ</i>	0	0	1	0	0/1	0
DN	4	1	11	2	5/13	38.5
Unaffected	1	1	10	5	2/15	13.3
Spouses	0	0	10	0	0/10	0

mutation; only five of 13 DN patients (38.5%) carried the Arg24Cys mutation. In contrast, all 12 invasive melanoma cases had this mutation. However, 10 of the 12 invasive melanoma patients had known DN status; all 10 had DN. Only one family member with melanoma *in situ* (and DN) did not have the Arg24Cys mutation. This individual was negative for a *CDKN2A* mutation. Thus, similar to what is seen in *CDKN2A* melanoma-prone families, the phenotype observed in melanoma-prone families with *CDK4* mutations appears to be more complex than just the mutation. Both genetic and environmental factors are likely to contribute to the occurrence of melanoma and DN in these families.

Direct assessment of the family members with the Arg24Cys mutation revealed a penetrance of approximately 63% (95% confidence interval 42–85%). Inclusion of the third melanoma-prone family with a co-segregating germline *CDK4* mutation, the French family with the Arg24His mutation,⁵ slightly reduced this estimate to 56% (95% confidence interval 37–74%). These estimates may be artificially inflated but are comparable to those found in families with *CDKN2A* mutations (unpublished data). The penetrance estimates for *CDK4* should be taken with caution though, since the median age of the non-penetrant mutation carriers (35 years; mean 37.7 years, SD 15.1 years in the Arg24Cys families) was similar to the median age at melanoma diagnosis (34.2 years; mean 38.9 years, SD 15.0 years for the 12 melanoma patients in the Arg24Cys families). Thus, some of the non-penetrant gene carriers may develop melanoma later in life.

Two melanoma susceptibility genes, *CDKN2A* and *CDK4*, have been identified to date.^{1–5} Mutations in *CDKN2A* have been frequently observed in melanoma-prone families from around the world. The frequency of detected mutations is directly related to the number of melanoma patients per family, with the frequency of mutations increasing with the number of melanoma patients in the family. The frequency of detectable mutations is less than 5% for

families with only two melanoma patients, whereas it increases to greater than 50% for families with more than six melanoma cases.^{2,9,10}

In contrast, co-segregating germline mutations in *CDK4* have been observed in only three kindreds worldwide.^{4,5} The original report of a germline *CDK4* mutation evaluated melanoma patients from 10 American and 21 Australian melanoma-prone families and found only one mutation, Arg24Cys, in two of the American kindreds.⁴ Thus, most subsequent studies of *CDK4* in melanoma-prone families have either exclusively tested for this alteration^{11,12} or have limited the evaluation to exon 2.^{5,10,13–16} Results from these studies revealed a single Australian kindred with a germline *CDK4* alteration, Ser52Asn.¹⁴ However, this novel sequence variant occurred in only two of the four melanoma patients in the kindred. Furthermore, the family also had a *CDKN2A* alteration (Ile49Ser) detected in three affected siblings but not in one affected first cousin. Thus, the significance of the *CDK4* variant remains unclear.¹⁴ In addition, a Danish study that analysed the entire coding sequence of *CDK4* in 56 sporadic metastatic malignant melanomas found a single novel missense mutation Asn41Ser in the tumour and germline of a patient who had no family history of melanoma.¹⁷ Whether this mutation contributes to the pathogenic phenotype of malignant melanoma also remains uncertain.¹⁷ Since overall only a small proportion of melanoma-prone families have been screened for all exons of *CDK4*, we screened 16 available *CDKN2A*-negative melanoma-prone families for alterations in all coding region exons of *CDK4*. No co-segregating mutations were found. Given the cumulative evidence, it seems very likely that, although *CDK4* is a melanoma susceptibility gene, it plays a minor role in hereditary melanoma.

The search for additional melanoma genes will be strongly influenced by the overall frequency of mutations in these genes. To date, for example, no mutations have been observed in several melanoma

susceptibility candidate genes, including *CDKN2B* (the gene that encodes the p15^{INK4B} proteins and is physically located adjacent to *CDKN2A*),^{12,18,19} *CDKN2C* (the gene that encodes the CDK inhibitor p18 located on chromosome 1p32),¹⁵ p19^{INK4D} (a member of the same INK4 family as p15 and p16),¹⁰ CDK6 (a CDK that complexes with cyclin D1 and is inhibited by p16),²⁰ and p53 (a tumour suppressor gene involved in cell cycle regulation).¹⁵ If the frequencies of additional melanoma susceptibility genes, including the ones for which no mutations have yet been detected, are comparable to the *CDK4* gene, they will be extremely difficult to identify. If, on the other hand, some of these genes have mutations with frequencies similar to that seen with *CDKN2A*, identification of these genes will be possible.

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