Multiple Melanomas After Treatment for Hodgkin Lymphoma in a Non-Dutch p16-Leiden Mutation Carrier With 2 MC1R High-Risk Variants

Adina Figl, MD; Ranjit K. Thirumaran, PhD; Selma Ugurel, MD; Andreas Gast, PhD; Kari Hemminki, MD; Rajiv Kumar, PhD; Dirk Schadendorf, MD

Background: A 19–base pair germline deletion in exon 2 of the CDKN2A (cyclin-dependent kinase inhibitor 2A) gene (Leiden mutation) has been detected in Dutch families with familial melanomas. The penetrance of CDKN2A mutations varies widely and is influenced by environmental and unrelated genetic factors such as variants in the MC1R gene.

Observations: We describe a 25-year-old German woman who developed 8 invasive melanomas and 6 in situ melanomas after radiation therapy and polychemotherapy for Hodgkin lymphoma. Genetic testing revealed a constitutional CDKN2A Leiden mutation in the proband and her sister, mother, and mother’s sister. The proband also carried high-risk MC1R variant alleles R151C and R160W, which she had inherited from her father and her mother, respectively. The less affected mutation carrier sister did not have high-risk MC1R variant alleles. Analysis of DNA from paraffin-embedded tissues showed loss of heterozygosity at CDKN2A loci in all 3 melanomas studied but not in Hodgkin lymphoma. The pedigree revealed several types of cancers on both sides of the family, but no Dutch ancestors were found. No mutations in the CDK4, B-raf, and N-ras genes were detected either in the germline or in tumors from the patient.

Conclusion: This study shows the variability of the penetrance of the CDKN2A Leiden mutation within the same family, which could be due to genetic or exogenous factors.

Arch Dermatol. 2007;143:495-499

Malignant melanoma is associated with complex genetic heterogeneity. An estimated 10% to 15% of melanomas occur in familial settings. Germline disease–segregating mutations in the CDKN2A (cyclin-dependent kinase inhibitor 2A) gene have been observed on average in up to 40% of melanoma-prone families. The penetrance of the CDKN2A mutations in carriers within melanoma families varies between 53% and 100% by the age of 80 years. However, a population-based survey has estimated an approximately 28% risk of melanoma in carriers of the CDKN2A mutation in the general population by the age of 80 years. The difference in penetrance rates between the latter study and previous studies is likely attributable to the different ascertainment approaches. However, variability in penetrance suggests risk modification by other genetic or environmental factors, including geographic location.

In Dutch families with familial atypical multiple mole melanoma, a founder germline 19–base pair (bp) frame-shift deletion has been described in exon 2 of the CDKN2A gene (Online Mendelian Inheritance in Man [OMIM] 600160.0003). All families with this particular deletion (named the CDKN2A Leiden mutation) were reported from the same geographic area in the Netherlands. It is presumed that all mutation carriers can be traced to a Dutch founder. Outside the Netherlands, only 4 families with the germline CDKN2A Leiden mutation have been described, to our knowledge. In 2003, a heterozygous mutation was detected in a blood sample from a 54-year-old man with 3 oropharyngeal tumors. Both of his parents and his only sister died of cancer when they were very young (the mother of gynecologic cancer, the father of liver carcinoma, and the sister of leukemia). Other melanoma-prone families with the segregating CDKN2A Leiden mutation have been reported from North America and Australia.
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In November 2004, a 24-year-old woman was referred to the skin cancer unit, Department of Dermatology, University Hospital of Mannheim, Mannheim, Germany. At the age of 20 years, she had been diagnosed as having axillary and mediastinal Hodgkin lymphoma, stage IIA, and received polychemotherapy with doxorubicin, bleomycin, vinblastine, and dacarbazine followed by radiation therapy. One year later, she again received radiation therapy for an early relapse and was in complete remission until June 2006, when she presented with a second relapse of Hodgkin lymphoma in both axillae. At the time of the writing of this article, she was consulting with her medical oncologists regarding possible high-dose chemotherapy.

Twenty-two months after the second radiation treatment, an ulcerated invasive melanoma (Breslow thickness 2.65 mm and Clark level IV) was excised from the patient’s left upper arm. An axillary sentinel lymph node biopsy showed micrometastasis, and complete lymph node dissection was performed, with no additional metastatic nodes. Within the following 2 months, 2 more invasive melanomas (Breslow thickness 0.41 mm and 0.56 mm) and 2 in situ melanomas were excised. After diagnosis of stage III melanoma, adjuvant low-dose subcutaneous interferon alfa therapy was initiated after an intravenous high-dose induction phase. However, half a year later, 4 more invasive melanomas (Breslow thickness between 0.35 and 0.50 mm) and 4 in situ melanomas were discovered and excised. The patient has lightly pigmented skin with a few freckles corresponding to skin phototype II, auburn hair, and a large number of nevi. Clinically, these nevi are small and regular rather than dysplastic.

**GENETIC TESTING**

The patient described an aunt (her mother’s sister) with a history of 4 invasive melanomas (Breslow thickness <1.0 mm), all clinically dysplastic. She received a total of 12 doses of interferon alfa-2b, with no clinical or histological evidence of disease, during the past 2 years of follow-up. The patient is currently undergoing interferon alfa-2b therapy for an early relapse of melanoma in situ after an intravenous high-dose induction phase. She is consulting with her medical oncologists regarding possible high-dose chemotherapy.

**METHODS**

Constitutional DNA samples were screened for mutations in the CDKN2A, CDK4, B-raf, and N-ras genes and for variants in the MC1R gene by direct DNA sequencing. For the CDKN2A gene, exons 1β, 1α, 2, and 3 were amplified using polymerase chain reaction (PCR) primers and conditions as described elsewhere. Similarly, exon 11 and 15 of the B-raf gene, exons 1 and 2 of the N-ras gene, and the MC1R gene were amplified using primers and conditions used previously. Amplified exons were sequenced using a commercially available cycle sequencing kit (BigDye Kit; Applied Biosystems, Foster City, Calif) and an automated sequencer (ABI 3100; Applied Biosystems). The LOH was scored if 1 of the 2 fragments determined by amplifying dinucleotide repeat microsatellite markers D9S974 and D9S942 using primers at the flanking sequences were electrophoresed under nondenaturating conditions, and mutations were detected by aberrant band shifts and sequencing.

**LOSS OF HETEROZYGOSITY**

Loss of heterozygosity (LOH) in paraffin-embedded tissues was determined by amplifying dinucleotide repeat microsatellite markers D9S974 and D9S942 using primers at the flanking sequences (www.gdb.org). Fluorescent-labeled PCR products were electrophoresed in an automated sequencer, and fragments were analyzed using fluorescent analysis software (Applied Biosystems). The LOH was scored if 1 of the 2 fragments in paraffin-embedded DNA from tumor tissue showed more than 50% reduction in size compared with corresponding constitutional DNA.
CDKN2A contained a heterozygous 19-bp deletion in exon 2 of the gene. Subsequently, other relatives were also screened for germline mutations in the CDKN2A gene. DNA from the 2 sisters and their mother and aunt contained a heterozygous 19-bp deletion in exon 2 of the CDKN2A gene, known as the CDKN2A Leiden mutation (Figure 2). No mutation was detected in any other investigated gene (Table). Furthermore, none of the other investigated relatives carried the mutation (Figure 1). Despite the 19-bp CDKN2A deletion being a founder mutation in the Netherlands, there have been no Dutch ancestors, going back at least 3 generations in the family under investigation.

The constitutional DNA from the family members was also genotyped for polymorphisms in the entire MC1R gene. The results showed that while the father was heterozygous for R151C and V60L polymorphisms; the mother carried the R160W variant allele. The previously unaffected sister inherited variant V60L from her father. However, the proband was heterozygous for high-risk R151C and R160W polymorphisms. The aunt (the CDKN2A Leiden deletion carrier) with a history of melanomas and ovarian cancer also carried the high-risk R151C variant allele (Table). A 57-year-old female cousin of the patient’s mother and aunt was homozygous for the variant I60W allele and was not a carrier of the CDKN2A Leiden mutation. The patient and her sister, mother, and aunt share a similar skin phenotype that clearly differs from other members of the family.

SOMATIC MUTATIONS AND LOH

Archival paraffin-embedded tissue was obtained from the Hodgkin lymphoma of the proband and from 3 of her invasive melanomas. All of these tissue samples carried the CDKN2A Leiden mutation. No mutation was detected in exons 11 and 15 of the B-raf gene or in exons 1 and 2 of the N-ras gene. Analysis of 2 microsatellite markers at the CDKN2A loci D9S942 and D9S974 in DNA from paraffin-embedded tissues showed LOH in all 3 melanoma samples at D9S942 and in 2 of the melanoma samples also at the D9S974 locus. No LOH was found in the Hodgkin lymphoma (Figure 2).

COMMENT

In this study, we detected a so-called CDKN2A Leiden mutation in the germline DNA of a 24-year-old patient who initially presented with Hodgkin lymphoma and who subsequently developed multiple melanomas after chemotherapy and radiation therapy. A similar analysis of DNA from her only sibling, a sister with no initial disease, showed her to be a mutation carrier. Both sisters had inherited the mutant CDKN2A allele from their malignancy-free mother. The mother’s sister, who had a history of multiple primary melanomas and ovarian cancer, was shown to be a mutation carrier as well. Because the other investigated relatives were noncarriers, we were not able to determine whether the mother and the aunt inherited the mutation from their mother or their father. The variability in the penetrance of the CDKN2A mutation in carriers implies a confounding effect of other genetic and environmental factors on phenotypic expression. This supposition is further supported by 2 known human homozygous p16-Leiden mutation carriers whose phenotype is similar to that of heterozygotes.*

We also found that the affected patient carried variant alleles for R151C and R160W polymorphisms in the MC1R gene.
gene, which were inherited from her father and mother. Incidentally, the other sister carried only the V60L variant allele. Interestingly, the affected aunt carried variant R151C, which the proband had inherited from her father. The father and aunt were not related. Variants of MC1R are known to contribute to melanoma risk in families with disease-segregating CDKN2A mutations. More than 65 human MC1R variants account for variations in skin and hair color and in skin cancer incidence by controlling the relative amounts of eumelanin and pheomelanin. Investigations in 16 American and 15 Australian CDKN2A families and in 6 Dutch p16-Leiden mutation families demonstrated that MC1R variants, notably the 3 red hair color variants R151C, R160W, and D294H, increased the penetrance of melanoma in mutation carriers.14-17 In the Dutch families, the MC1R-melanoma association was primarily related to the R151C variant. The concurrent occurrence of the high-risk MC1R allele and the CDKN2A Leiden mutation might be the reason that the proband and her aunt were more seriously affected than the patient’s sister and mother. However, at the same time, immunosuppression due to Hodgkin disease could also be the reason for multiple melanomas in the proband. Another possible cause could be the doxorubicin, bleomycin, vinblastine, and dacarbazine treatment of Hodgkin disease.

The analysis of tumors from the patient with lymphoma and multiple melanomas showed loss of the wild-type allele of CDKN2A in melanomas but not in lymphomas. The wild-type allele of a tumor suppressor is lost through disjunctional chromosomal loss, or gene conversion, with melano- rubicin, bleomycin, vinblastine, and dacarbazine treatment of Hodgkin disease.

Table. Constitutional Mutations in the CDKN2A, CDK4, B-raf, N-ras, and MC1R Genes in the Family of a Proband With Multiple Melanomas

<table>
<thead>
<tr>
<th>Gene</th>
<th>Patient</th>
<th>Sister</th>
<th>Mother</th>
<th>Father</th>
<th>Aunt</th>
</tr>
</thead>
<tbody>
<tr>
<td>B-raf</td>
<td>WT</td>
<td>WT</td>
<td>WT</td>
<td>WT</td>
<td>ND</td>
</tr>
<tr>
<td>N-ras</td>
<td>WT</td>
<td>WT</td>
<td>WT</td>
<td>WT</td>
<td>ND</td>
</tr>
<tr>
<td>CDK4</td>
<td>WT</td>
<td>WT</td>
<td>WT</td>
<td>WT</td>
<td>ND</td>
</tr>
<tr>
<td>MC1R</td>
<td>R151C and R160W</td>
<td>V60L</td>
<td>R160W</td>
<td>R151C and V60L</td>
<td>R151C</td>
</tr>
</tbody>
</table>

Abbreviations: CDKN2A, cyclin-dependent kinase inhibitor 2A; ND, not done; WT, wild type.

were detected. Somatic mutations in the B-raf gene are of common occurrence in melanomas; however, germ-line mutations in the gene are rare.12,22 Somatic mutations in the N-ras gene complement mutations in the B-raf gene and occur to a certain extent in melanomas. In an earlier study, tumors from patients belonging to melanoma families and carrying the CDKN2A mutation showed a high frequency of N-ras mutations.23 The ras-raf mitogen–activated protein kinase pathway interferes with the cellular response to cyclic adenosine monophosphate–dependent growth signals in melanocytes. However, it is possible that increased melanocytic proliferation due to a germline CDKN2A mutation abrogates requirement for mutations in the B-raf and N-ras genes.

Leiden mutation carriers are also at an estimated risk of 17% to develop pancreatic cancer by the age of 75 years, although pancreatic cancer was observed in only 4 of the investigated 19 families with this specific mutation.19 The association of the pancreatic cancer of our proband’s granduncle with the CDKN2A Leiden mutation remains unknown. However, based on our findings, we conclude that all mutation carriers should be advised to undergo regular clinical checkups for melanoma and pancreatic cancer.

Accepted for Publication: April 26, 2006.

Correspondence: Dirk Schadendorf, MD, Skin Cancer Unit, German Cancer Research Center Heidelberg and Department of Dermatology, University Hospital of Mannheim, Theodor-Kutzer-Ufer 1, D-68167 Mannheim, Germany (d.schadendorf@dkfz.de).

Author Contributions: Study concept and design: Figl, Ugurel, Kumar, and Schadendorf. Acquisition of data: Figl, Thirumaran, Gast, Kumar, and Schadendorf. Analysis and interpretation of data: Figl, Ugurel, Hemminki, Kumar, and Schadendorf. Drafting of the manuscript: Figl, Kumar, and Schadendorf. Critical revision of the manuscript for important intellectual content: Thirumaran, Ugurel, Gast, Hemminki, Kumar, and Schadendorf. Obtained funding: Schadendorf. Administrative, technical, and material support: Figl, Thirumaran, Ugurel, Gast, Kumar, and Schadendorf. Study supervision: Hemminki, Kumar, and Schadendorf.

Financial Disclosure: None reported.

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