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

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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.

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METHODS

MUTATION ANALYSIS IN CONSTITUTIONAL DNA

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. Exon 11 and 15 of the B-raf gene, exon 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).

MUTATION ANALYSIS OF TUMOR DNA FROM PARAFFIN-EMBEDDED TISSUES

Mutations in DNA from paraffin-embedded tissues were detected by single-strand conformation polymorphism using PCR products with a phosphorus 32–labeled radioisotope incorporated during amplification reactions. Radiolabeled PCR products 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 fragment 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.

RESULTS

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

Figure 1. Pedigree representing a family with a CDKN2A (cyclin-dependent kinase inhibitor 2A) 19–base pair deletion. Numbers outside parentheses represent current age or age at death, as appropriate, in years. Numbers within parentheses give age at onset of cancer, also in years. The proband is indicated by an arrow. The father of the proband’s mother died of a septicemia of unknown origin at the age of 39 years. Circles indicate females; squares, males; diagonal lines, deceased; shaded central dots, p16-Leiden mutation; black outer circles, melanoma; vertical lines, no Leiden mutation; and open circles and squares, mutation status unknown.
mm), 1 melanoma in situ, and ovarian cancer. Because of her unusual personal and family melanoma history, we suggested genetic testing for alterations at melanoma susceptibility loci. During these investigations, the patient developed 1 more invasive melanoma (Breslow thickness 0.6 mm), and her sister was diagnosed for the first time as having melanoma in situ. Neither of the parents has presented with malignancy so far, but reconstruction of a pedigree revealed multiple cancers on both sides of the family (Figure 1). Constitutional DNA from the patient, her only sibling sister, and their parents was screened for mutations in the CDKN2A, CDK4, B-raf, and N-ras genes. 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
Table. Constitutional Mutations in the CDKN2A, CDK4, B-ras, 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-ras</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>
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Abbreviations: CDKN2A, cyclin-dependent kinase inhibitor 2A; ND, not done; WT, wild type.

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. 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, vindristine, 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 mostly through deletion, mitotic recombination, non-disjunctional chromosomal loss, or gene conversion, termed LOH. However, silencing of the wild-type allele through promoter methylation has also been reported. In a more specific instance, in a case of a p16-Leiden mutation carrier with head and neck cancer, the wild-type allele showed promoter methylation in a tumor.

The attribution of Hodgkin lymphoma to the CDKN2A mutational status is elusive, although loss of p16 expression in Hodgkin and Reed-Sternberg cells as well as non-Hodgkin lymphomas in CDKN2A mutation carriers and leukemia in a first-degree relative of a carrier have been reported. To date, no report, to our knowledge, has shown Hodgkin lymphoma in CDKN2A Leiden mutation carriers. Furthermore, a strong statistical association has been reported between the occurrence of non-Hodgkin lymphoma and cutaneous melanoma.

Although we analyzed both the B-ras and the N-ras genes in the germline as well as in tumors from a patient with lymphoma and multiple melanomas, no mutations were detected. Somatic mutations in the B-ras gene are of common occurrence in melanomas; however, germline mutations in the gene are rare. Somatic mutations in the N-ras gene complement mutations in the B-ras 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. The ras-ras mi- togen–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-ras 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. 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.

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REFERENCES


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