Pancreatic Carcinoma Surveillance in Patients With Familial Melanoma

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Objective: To determine the optimal methods for pancreatic adenocarcinoma surveillance in high-risk patients with familial melanoma and cyclin-dependent kinase inhibitor 2A (CDKN2A) mutations.

Design: Case report with pedigree analysis and literature review, with an emphasis on guideline development for high-risk kindreds with familial pancreatic adenocarcinoma.

Setting: A university-affiliated familial melanoma research clinic.

Patients: The proband was referred as a participant in a research clinic protocol and was found to carry a germ-line CDKN2A mutation and have a history of melanoma and pancreatic adenocarcinoma. A total of 179 family members were identified through the Utah Population Database and underwent evaluation for history of melanoma and pancreatic adenocarcinoma.

Intervention/Methods: Comprehensive family history and pedigree analysis performed by means of personal interview, medical record review, and use of cancer registry and population database records. Mutation status was confirmed by results of DNA sequence analysis. Tumor identity was confirmed with immunohistochemical markers.

Main Outcome Measures: Estimated risk for pancreatic adenocarcinoma in a high-risk family with CDKN2A-positive melanoma. Guidelines for surveillance in these families were based on review of the literature.

Results: Sequence analysis confirmed a CDKN2A mutation, and immunohistochemical evaluation confirmed the diagnoses of metastatic melanoma and metastatic pancreatic adenocarcinoma. Pedigree analysis showed an observed-expected ratio of 8.9 to 12.6 for pancreatic adenocarcinoma and 16.4 to 20.8 for melanoma in this family. Guidelines used for surveillance of kindreds at high risk for pancreatic adenocarcinoma were applied to families with CDKN2A melanoma.

Conclusion: Patients with melanoma and a germline CDKN2A mutation should be considered for pancreatic adenocarcinoma surveillance that is based on the most recent published studies.

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T has been estimated that approximately 5% to 12% of melanomas occur in the familial setting. Genetic linkage studies and molecular analyses have established cyclin-dependent kinase inhibitor 2A (CDKN2A, p16, and INK4a) as the major melanoma susceptibility gene in the familial melanoma syndrome. Mutations in the CDKN2A gene allow cells to bypass the G1/S cell cycle checkpoint, leading to uncontrolled cell proliferation and growth. Mutations in the CDKN2A gene are found in approximately 20% to 40% of melanoma-prone families and are associated with the familial atypical multiple mole melanoma syndrome (FAMMM). Growing evidence suggests that CDKN2A mutations may also predispose patients to pancreatic carcinoma. A study of Dutch families with melanoma by Vasen et al identified a specific 19-base-pair (bp) deletion in exon 2 of the CDKN2A gene, which they have named the p16-Leiden deletion, in 19 families with FAMMM. The second most frequent cancer in these families, after melanoma, was pancreatic carcinoma. The cumulative risk for development of pancreatic cancer in individuals with the p16-Leiden deletion was 17%, with a mean age at diagnosis of 58 years, whereas no pancreatic carcinoma developed in individuals from families with suspected FAMMM without the p16-Leiden deletions. However, families with other CDKN2A mutations were not studied. A study of Dutch FAMMM families by Hille et al showed nearly equal mortal-
appropriately owing to an unacceptable rate of false-screening tests for the general population are not difficult to screen for pancreatic carcinoma, and approximately 50% of any cancer.28 Approximately 50% Rb/p16
CDKN2A
2 malignancies are so often seen together in patients with carcinoma in the same patient is evidence enough to virtually prove that the patient has an underlying germline CDKN2A mutation. Those authors recommend that relatives of individuals with these double primary malignancies obtain careful screening for melanoma. Vasen et al17 have also recommended surveillance for pancreatic cancer in families with FAMMM in the setting of a prospective study.

Pancreatic carcinoma is the fourth leading cause of cancer death in the United States,28,29 It has the lowest 5-year survival rate of any cancer.29 Approximately 50% of patients have metastatic disease at diagnosis,28,30 It is difficult to screen for pancreatic carcinoma, and screening tests for the general population are not appropriate owing to an unacceptable rate of false-positive findings.30

We herein present the case of a patient from a kindred with familial melanoma and an established functional germline CDKN2A mutation. He survived malignant melanoma with widespread metastases, but died of metastatic pancreatic adenocarcinoma 25 years after his initial melanoma diagnosis. We reviewed the most recent literature surrounding screening methods for early detection of pancreatic tumors, with the goal of evaluating the role of traditional and more innovative surveillance methods in patients with familial malignant melanoma and CDKN2A mutations.

**REPORT OF A CASE**

The patient first presented in 1978 at 32 years of age with a clinically atypical melanocytic lesion on the right side of his back. The lesion was excised, and pathological evaluation confirmed superficial spreading malignant melanoma, with a depth of 1.24 mm, or Clark level IV. He underwent a wide local excision 6 months later, and results of pathological evaluation showed no further evidence of melanoma in the surrounding tissue. Fourteen months after his initial diagnosis, at 33 years of age, he presented with painful right axillary lymphadenopathy. Right axillary lymph node dissection was performed, and histological sections revealed metastatic malignant melanoma. One month later, he was referred for local radiation therapy of his right axilla. Staging revealed several pulmonary nodules in the right lung. The patient underwent segmental resection of the right upper, middle, and lower lobes, and pathological analysis confirmed metastatic melanoma. After resection, several pulmonary nodules remained, and new ones appeared within 2 months of surgery. The patient underwent 21 courses of chemotherapy with dacarbazine and dactinomycin (actinomycin D) during the course of 2 years. He had complete regression of metastatic melanoma, with no evidence of residual disease.

Two years after chemotherapy, at 39 years of age, the patient had a new primary melanoma (Clark level I-II) on his left shoulder, which was excised. Another primary melanoma, 0.57 mm in depth (Clark level III), was excised from his left upper back 7 years later, at 46 years of age. Both of these lesions were contiguous with the epidermis, suggesting that they were new primary lesions rather than metastatic in origin. In addition, he was diagnosed as having numerous dysplastic nevi, atypical melanocytic hyperplasia, and benign compound nevi.

At 57 years of age, the patient was enrolled in the Familial Melanoma Research Clinic at the Huntsman Cancer Institute at the University of Utah, Salt Lake City. Sequence analysis of DNA showed that he was heterozygous for a V126D (aspartate substituted for valine at amino acid 126 CDKN2A mutation) (Figure 1). He had a family history of melanoma and numerous atypical nevi, as well as pancreatic carcinoma (Figure 2), and was diagnosed as having FAMMM syndrome. This pedigree was evaluated using the familial standardized incidence ratio to establish observed-expected ratios for melanoma and pancreatic cancer (Figure 2).31 Two months later, the patient presented to his primary care physician complaining of chronic low back and abdominal pain. Magnetic resonance imaging showed a 2.4-cm enhancing mass in the right kidney. Computed tomography of the abdomen and pelvis showed a 3.3 × 3.2-cm hypoechocic mass in the right kidney and ascites surrounding the liver and spleen. Results of cytology of a paracentesis specimen were consistent with adenocarcinoma, and results of an immunohistochemistry evaluation were negative for melanoma markers (vimentin, S100, and HMB-45) and positive for mucin (Figure 3). This finding was not consistent with renal cell carcinoma or metastatic melanoma. A colonoscopy was performed, but results of all biopsies were negative. Levels of diagnostic serum tumor markers carcinoembryonic antigen, β-human chorionic gonadotropin, and α-fetoprotein were all within reference range, suggesting that the tumor was less likely of colorectal, hepatic, or germ cell origin. However, the cancer antigen 19-9 (CA 19-9) level was markedly elevated to 17 293 U/mL (reference range, 0-37 U/mL). The CA 19-9 result, in conjunction with the negative results of other studies, led to a presumptive diagnosis of pancreatic adenocarcinoma. Laparoscopic exploration revealed widespread metastases. Tissue from the falciform...
ligament and omentum were positive for cytokeratin 7, carcinomaembryonic antigen, and epithelial membrane antigen, and negative for cytokeratin 20, a pattern consistent with pancreatic carcinoma (Figure 3).32-35 The patient was discharged to hospice care following the laparoscopy and died 11 days later.

**SEQUENCING AND ANALYSIS OF GENOMIC DNA**

Genomic DNA was isolated from blood using a commercially available kit (Puregene DNA Isolation Kit; Gentra Systems, Inc, Minneapolis, Minn). Polymerase chain reaction (PCR) products were generated for sequence analysis, including the primer region and exons 1, 2, and 3 of CDKN2A, spanning the splice junctions. Primers were synthesized by means of the University of Utah DNA/Peptide core facility, Salt Lake City, and the sequences were as follows (5' to 3'): distal promoter region (forward, 5'–GCGAGCAAAGCGGTGTTT–3'); reverse, 5'–GCCAGCAAAGCGGTGTTT–3'); proximal promoter region (forward, 5'–GCCATCTTTTCCCTATGACAC–3'); reverse, 5'–GCCTCACTCTCTCTATGACAC–3'); exon 1 (forward, 5'–CACCTCTCCCGAGACACTC–3'); reverse, 5'–CTCACTCTCCCTCTCTATGACAC–3'); exon 2 (forward, 5'–GACGACAGCTCGAGGAAGTTA–3'); reverse, 5'–GGTGAAGCTGAGGCAAGACG–3'); and exon 3 (forward, 5'–GTGGAAGGACGCCAGAAGGA–3'); reverse, 5'–TGAAATAGGTGCTCATAAAAATGTGACA–3'). Polymerase chain reactions were performed using 0.5 µm forward and reverse primers, 1 x PCR buffer (Idaho Technology Inc, Salt Lake City, Utah); 0.25 mM deoxynucleoside triphosphates (Takara Bio Inc, Otsu, Japan), 50- to 100-ng genomic DNA template, and 1.0-U Taq DNA polymerase (Fermentas Inc, Hanover, Md) with Taq antibody (BD Biosciences Clontech, Palo Alto, Calif). Cycling conditions included an initial 5-minute incubation at 94°C, followed by 40 cycles of 94°C for 30 seconds of denaturing, 58°C for 30 seconds of annealing, and 72°C for 60 seconds of extension. A final extension period of 5 minutes at 72°C was performed at completion of the 40 cycles. Before sequencing, each sample was treated with exonuclease I and shrimp alkaline phosphatase (EXOSAPIT; USB Corporation, Cleveland, Ohio). Sequencing was performed using the same primers by the University of Utah Sequencing Core Facility with a fluorescent capillary sequencer (3700 AB1; Applied Biosystems, Foster City, Calif).

**IMMUNOHISTOCHEMISTRY**

Histological analysis and immunohistochemistry were performed as previously described.36-37 Briefly, formalin-fixed, paraffin-embedded tissue sections were deparaffinized in xylene and absolute ethanol, then rehydrated by means of successive immersions in 95% ethanol, 70% ethanol, and distilled water. Immunoperoxidase staining was performed on the Ventana ES instrument (Ventana Medical Systems, Inc, Tucson, Ariz) using the avidin-biotin-peroxidase complex according to manufacturer recommendations. We applied primary antibodies to proteins S100 (DAKO Corporation, Carpinteria, Calif) diluted 1:700, cytokeratin AE1/AE3 (Boehringer Mannheim Biochemicals, Indianapolis, Ind) diluted 1:1400, and Melan-A (DAKO Corporation) diluted 1:20 (heat-induced epitope retrieval was performed before primary antibody incubation). The immunostaining was developed using diaminobenzidine as the chromogen, and samples were counterstained with Mayer hematoxylin. We performed hematoxylin-eosin, mucicarmine, and Wright stains according to standard laboratory protocols.

**PEDIGREE ANALYSIS**

Pedigree analyses were performed through the use of the Utah Population Database. This database contains computerized genealogies from the Family History Library of the Church of
Figure 3. Histological and immunohistochemical differences between the patient’s melanoma and pancreatic carcinoma. A, Metastatic melanoma of the lung (hematoxylin-eosin, original magnification ×200). Sections of the lung mass demonstrate a poorly differentiated neoplasm composed of pleomorphic epithelioid cells arranged in variably sized nests. B, Pancreatic adenocarcinoma (hematoxylin-eosin, original magnification ×200). Sections of the pancreatic mass demonstrate a cytologically malignant neoplasm forming glands with focal mucin production. C, Metastatic melanoma of the lung (S100, original magnification ×100). Neoplastic cells are immunoreactive for S100 but not for Melan-A (DAKO Corporation, Carpinteria, Calif; diluted 1:20) (E, original magnification ×200), HMB-45 (DAKO Corporation; diluted 1:50) (not shown), or cytokeratin AE1/AE3 (G, original magnification ×200). D, Pancreatic adenocarcinoma (S100, original magnification ×100). Neoplastic cells are not immunoreactive with S100 but are strongly positive for cytokeratin AE1/AE3 (F, original magnification ×200). H, Aspirate of peritoneal fluid reveals clusters of malignant cells forming glandular lumina (Wright stain, original magnification ×200). Findings of mucicarmine stain are positive within tumor aggregates (inset [right portion of H], original magnification ×200).
The involvement of CDKN2A in susceptibility to melanoma is now well established. The estimated gene penetrance for melanoma in those with CDKN2A mutations by 80 years of age is 58% to 92% and is geographically variable. After melanoma, pancreatic carcinoma is the cancer most commonly associated with the familial melanoma syndrome and CDKN2A mutations. Penetrance estimations for pancreatic carcinoma in those with CDKN2A mutations have not yet been well established, but a Dutch study estimated a 17% penetrance by 75 years of age in FAMMM families with the p16-Leiden 19-bp deletion. Despite these high penetrance numbers and the fact that 20% to 40% of familial melanoma is associated with CDKN2A mutations,1,2,12 most clinicians take no precautions to prevent this fatal disease.

It is well known that pancreatic cancer is an extremely aggressive tumor, and early detection is exceptionally difficult. Barriers to early detection include absence of symptoms at early stages of disease and the retroperitoneal placement of the pancreas. The most common presenting symptoms of pancreatic cancer include chronic epigastric pain radiating to the back, jaundice, weight loss, diarrhea, steatorrhea, and new-onset diabetes or hyperglycemia.28,40-42 Once diagnosed, pancreatic cancer is notoriously difficult to treat. It is generally diagnosed after it has metastasized, and patients usually die within 12 months of diagnosis. To date, no known screening method is sensitive and specific enough to screen the asymptomatic general population. Experts suggest that surveillance is warranted in high-risk populations, such as those with a family history of pancreatic cancer or melanoma, but optimal screening methods are uncertain.17,27,30,41,43 Although effective strategies have been developed to screen for early detection of breast, prostate, colon, and cervical cancer, the pancreas creates an obstacle for similar screening methods because of the difficulty in visualizing or obtaining tissue from this organ.

In the past, traditional imaging techniques have been the primary focus of research in this area. The American Gastroenterological Association recommends at-risk patients undergo screening with spiral computed tomography, endoscopic ultrasonography (EUS), and measurement of CA 19-9 level as the initial screening tests. More recent work has found EUS and endoscopic retrograde cholangiopancreatography (ERCP) to have sensitivity and specificity values in the lower to middle 90s for diagnosis of pancreatic carcinoma.12,44,46 The major limitation of EUS and ERCP is their invasiveness. Patients require conscious sedation for the procedure and have about a 5% risk for complications, including acute pancreatitis and hemorrhage.46,47 Although these imaging studies appear to be promising for the diagnosis of pancreatic carcinoma, their utility as a screening or surveillance method is uncertain. Brentnall has suggested an algorithm for surveillance of those at high risk for pancreatic cancer, beginning 10 years before the earliest diagnosis of pancreatic carcinoma in the patient’s family or 50 years of age, whichever comes first.

Another promising modality for surveillance is the measurement of tumor markers, which are easily detected in body fluids and tissues. At present, there are no known tumor–specific markers for pancreatic cancer, although CA 19-9 is widely used.48,49 With a cutoff of 37 U/mL, the CA 19-9 level has sensitivity of 81% to 85% and specificity of 81% to 90% in detecting pancreatic adenocarcinoma.50,51 Steinberg has shown that CA 19-9 levels greater than 1000 U/mL approach 100% specificity for diagnosing pancreatic cancer. However, in 1 study, serum CA 19-9 and carciinoembryonic antigen levels were not shown to be sensitive enough markers for early detection of premalignant lesions.42 Although measurement of CA 19-9 levels may not be sensitive enough to detect pancreatic dysplasia, changes in CA 19-9 levels over time may be sensitive enough to detect early pancreatic adenocarcinoma, perhaps before the tumor has metastasized or grown large enough to be resectable. Sensitivity and specificity of 80% to 90%, as estimated for CA 19-9 levels in detection of pancreatic cancer, may not be adequate for screening in the asymptomatic population. However, in high-risk patients such as those with CDKN2A mutations, these statistics suggest that an annual measurement of this antigen level may be beneficial as surveillance.

To improve screening methods for early detection of pancreatic carcinoma, a better understanding of the molecular and histological pathology involved in progression of this cancer is needed. A model outlining the progression of pancreatic cancer has recently been introduced by Hruban et al. This model was patterned after the genetic progression model for colorectal neoplasia. The model proposes that pancreatic cancer progresses from normal ductal epithelium to ductal hyperplasia to invasive ductal adenocarcinoma. The authors use the term pancreatic intraepithelial neoplasia to describe this progression from normal epithelium to differing levels of neoplasia. In sporadic pancreatic carcinoma, the progression from level 1 to level 3 disease is often associated with a sequence of genetic alterations, first in K-ras and Her-2/neu, followed by p16 alterations, and finally abnormalities in p53, DPC4, BRCA2, and other tumor suppressor genes. Genetic changes in oncogenes, tumor suppressors, and telomerase are being studied as noninvasive screening tests in individuals at risk for development of pancreatic cancer. Testing of stool, pancreatic juice, and blood for K-ras, p53, p16, and DPC4 mutations or telomerase activity is still in its infancy, but it is hoped that highly sensitive methods such as restriction fragment length polymorphism PCR will soon be available for serological testing. Further study in these areas is warranted before these methods can be used as a surveillance method for pancreatic carcinoma.
We propose an algorithm to be used for surveillance of patients with familial melanoma and known CDKN2A mutations, based on the surveillance program recommended by Brentnall et al. in 2000 (Figure 4). Although no screening method has been effective in reducing the mortality rate for sporadic pancreatic cancer, patients with a known CDKN2A mutation are at high risk and surveillance is appropriate in this subpopulation. Confirmed CDKN2A mutations are present in only 20% to 40% of families with familial melanoma, and there is no known or proven correlation between families with non-CDKN2A familial melanoma and increased predisposition to pancreatic cancer. Therefore, we cannot recommend pancreatic cancer surveillance on any member of a kindred with FAMMM without a known CDKN2A mutation. At present, CDKN2A results are obtained primarily through research protocols, making referral to one of these studies important for these family members.

Surveillance should begin 10 years before the age at onset of pancreatic carcinoma in the youngest member of the patient’s family who has been diagnosed as having pancreatic carcinoma. If no family history of pancreatic carcinoma is known, surveillance should begin at 50 years of age. Initial screening with EUS is suggested in asymptomatic patients. The annual EUS examination may be appropriate in those with a known CDKN2A mutation or familial melanoma. If the annual EUS examination is abnormal, ERCP will be performed. If the patient is symptomatic or has a history of pancreatitis, alcohol abuse, or cholelithiasis, which can influence the EUS findings, ERCP should be performed initially in addition to the EUS. If suspicious changes are detected on ERCP, total pancreatectomy should be considered. Spiral computed tomography should be considered before surgery to detect large masses or metastases. Frozen sections should be obtained in the operating room and evaluated by a pathologist who is a pancreatic specialist to make a histological diagnosis of pancreatic dysplasia or carcinoma before the pancreatectomy. Such a surveillance program is best performed at a center specializing in the endoscopy, pathology, and surgery of the pancreas.

At present, the CA 19-9 level is not used as a screening method for high-risk patients or those with sporadic disease because of concern regarding insensitivity and lack of specificity in sporadic pancreatic cancer. However, in certain high-risk subsets of patients, annual testing in conjunction with EUS may increase the capacity for early detection. We suggest an annual measurement of the CA 19-9 level. If a patient is found to have a CA 19-9 level near or greater than 37 U/mL or experiences a dramatic increase in the level during a 1-year period, ERCP is suggested. Until K-ras, p53, or DPC4 mutations or telomerase levels (or a combination of these tests) have been validated as screening tools, these are not recommended outside research protocols. In the future, we envision a screening test for patients with familial melanoma and CDKN2A mutations using an annual quantitative serum measurement of CA 19-9, carcinoembryonic antigen, K-ras, or telomerase level to detect early pancreatic neoplasm, much as the prostate-specific antigen level is used to detect prostate cancer. Future research on the cost-benefit ratio and efficacy of these screening recommendations in families with the CDKN2A mutation is planned. If benefit is demonstrated, clinical CDKN2A testing in families with familial melanoma may be warranted.

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