

BRIEF REPORT: A FAMILIAL SYNDROME OF PANCREATIC CANCER AND MELANOMA WITH A MUTATION IN THE *CDKN2* TUMOR-SUPPRESSOR GENE

ALISON J. WHELAN, M.D., DETLEF BARTSCH, M.D.,
AND PAUL J. GOODFELLOW, PH.D.

THE distinguishing characteristics of familial cancer syndromes are an inherited predisposition to one or more characteristic types of tumors, early age at onset, and multiple synchronous or asynchronous tumors. Recently, the genes responsible for a number of inherited cancers have been identified.¹⁻⁵

We describe a kindred with an increased risk of pancreatic cancers, melanomas, and possibly additional types of tumors. We provide strong evidence linking the predisposition to cancer in this kindred to an inherited mutation in the cyclin-dependent-kinase inhibitor 2 (*CDKN2*) tumor-suppressor gene. The results of our study of this family suggest that disruption of the function of *CDKN2* contributes to pancreatic tumorigenesis.

CASE REPORTS

Squamous-cell carcinoma of the tongue developed in a 34-year-old woman (the proband). She did not drink alcohol or use tobacco. She was referred for genetic evaluation because of the early age of onset in the absence of known risk factors and a strong family history of pancreatic carcinoma. A detailed family history revealed that two other family members had also had malignant melanomas. The proband's mother (Subject III-3) had died of metastatic pancreatic carcinoma at the age of 57 (Fig. 1). Pathological examination of the tumor revealed squamous-cell carcinoma, a rare form of pancreatic carcinoma that accounts for approximately 2 percent of all such carcinomas.⁶ The proband's maternal aunt (Subject III-2) died of metastatic adenocarcinoma of the pancreas at the age of 45. The proband's maternal uncle (Subject III-1) died of metastatic melanoma at the age of 32. Her maternal grandmother (Subject II-2), who had localized spread of melanoma at the age of 70, died of metastatic adenocarcinoma of the pancreas at the age of 73. An experienced pathologist confirmed the diagnosis of pancreatic cancer in these three family members by reviewing representative slides. The proband's father and sisters are healthy.

Examination of the pedigree raised the question whether the pancreatic cancer and the melanoma were clinical expressions of a familial cancer syndrome. The unusual finding of a squamous-cell carcinoma of the pancreas in one family member and the early onset of the squamous-cell carcinoma of the tongue in the proband were also consonant with a familial cancer syndrome. A likely candidate gene in this family was *CDKN2*, which encodes p16. This protein inhibits cyclin-dependent kinase 4, thereby regulating the cell cycle.⁷

METHODS

Preparation of Genomic DNA and Characterization of *CDKN2* Sequences

DNA was prepared from formalin-fixed, paraffin-embedded specimens of tumor tissue⁸ from Subjects II-2, III-2, and III-3 (Fig. 1). The only tissue available from Subject II-2 was a transduodenal needle-biopsy specimen of a poorly differentiated pancreatic adenocarcinoma, for which the neoplastic cellularity was estimated as being 40 percent. In the case of Subject III-2, two needle-biopsy specimens of the moderately differentiated pancreatic adenocarcinoma were available.

From the Departments of Medicine and Pediatrics (A.J.W.) and Surgery (D.B., P.J.G.), Washington University School of Medicine, St. Louis, and the Department of Surgery, Philipps University, Marburg, Germany (D.B.). Address reprint requests to Dr. Goodfellow at Box 8109, 660 S. Euclid, St. Louis, MO 63110.

Supported in part by a grant (Ba 1467/1-1) from the Deutsche Forschungsgemeinschaft (to Dr. Bartsch).

The neoplastic cellularity of the tumor was estimated at only 5 percent in one of the specimens and at 50 percent in the other. The biopsy specimen from Subject III-3 was determined to contain approximately 30 percent neoplastic cells. The DNA prepared from the archival tumor-tissue specimens represented a mixture of normal cellular (constitutional) DNA and cancer-cell DNA. On the basis of the neoplastic-cell content of each of the specimens, the constitutional DNA made up an estimated 60 percent of the specimen from Subject II-2, 95 percent of one of the specimens from Subject III-2, and 70 percent of the specimen from Subject III-3. Thus, there was sufficient constitutional DNA in the tissue specimens to allow us to examine the germ-line *CDKN2* gene in the family members. The low neoplastic cellularity of the available tumor-tissue specimens made studies of the loss of heterozygosity and the search for tumor-specific mutations of *CDKN2* impractical. No tissue samples were available from Subject I-1, I-2, or III-1. Subject II-1 chose not to participate in the study. DNA was extracted from peripheral-blood samples from Subjects III-4, IV-1, IV-2, and IV-3.⁹

DNA from family members was evaluated for alterations in *CDKN2* by single-strand conformational-variant (SSCV) and DNA-sequence analysis. The primers and conditions used for SSCV analysis were as described previously,^{10,11} with the following modification. For amplicon B of exon 2, new primers were devised to improve the reliability of the polymerase-chain-reaction (PCR) assay. The forward primer was 5'AACTGCGCCGACCCCGCCACT3', and the reverse primer was 5'TCAGCCAGTCCACGGGAGAG3'. Variant bands were excised from dried SSCV gels, reamplified, and sequenced directly.¹¹ Sequencing was performed on both DNA strands (sense and antisense), and all reactions were repeated at least twice. For all family members investigated, DNA sequencing was performed for amplicon B of exon 2.

DNA-Marker Genotyping Studies

The (CA)_n microsatellite-repeat markers at D9S157 and D9S171 flanking the *CDKN2* locus were typed for all members of the kindred as described previously.¹¹

RESULTS

An SSCV variant was identified in exon 2 (amplicon B) of the *CDKN2* gene in the constitutional DNA of the proband (Subject IV-1). Direct sequencing of the variant revealed a G→T nucleotide change at position 295, resulting in the substitution of a tryptophan residue for glycine at amino acid position 93 (Gly93Trp) (Fig. 2). Sequence analysis of the PCR-amplified *CDKN2* gene from three additional family members (Subjects II-2, III-2, and III-3), all of whom had died of pancreatic cancer, showed that they too had the Gly93Trp mutation. In each case, the Gly93Trp mutation was present along with the wild-type (normal) *CDKN2* sequence. The proband's unaffected father and two siblings did not carry the *CDKN2* mutation. Analysis of the pattern of inheritance of the polymorphic DNA markers D9S171 and D9S157 that are linked to *CDKN2* demonstrated that within the kindred the *CDKN2* mutation was associated with a single DNA haplotype. All the affected family members shared both the Gly93Trp mutation and specific alleles at D9S171 and D9S157, whereas unaffected family members had neither the *CDKN2* mutation nor the pattern of polymorphism at D9S171 and D9S157 that cosegregated with disease (data not shown).

DISCUSSION

We studied a family in which a *CDKN2* mutation cosegregated with several types of cancer. Several lines of evidence suggest that the *CDKN2* mutation is present in the constitutional DNA of all the affected family

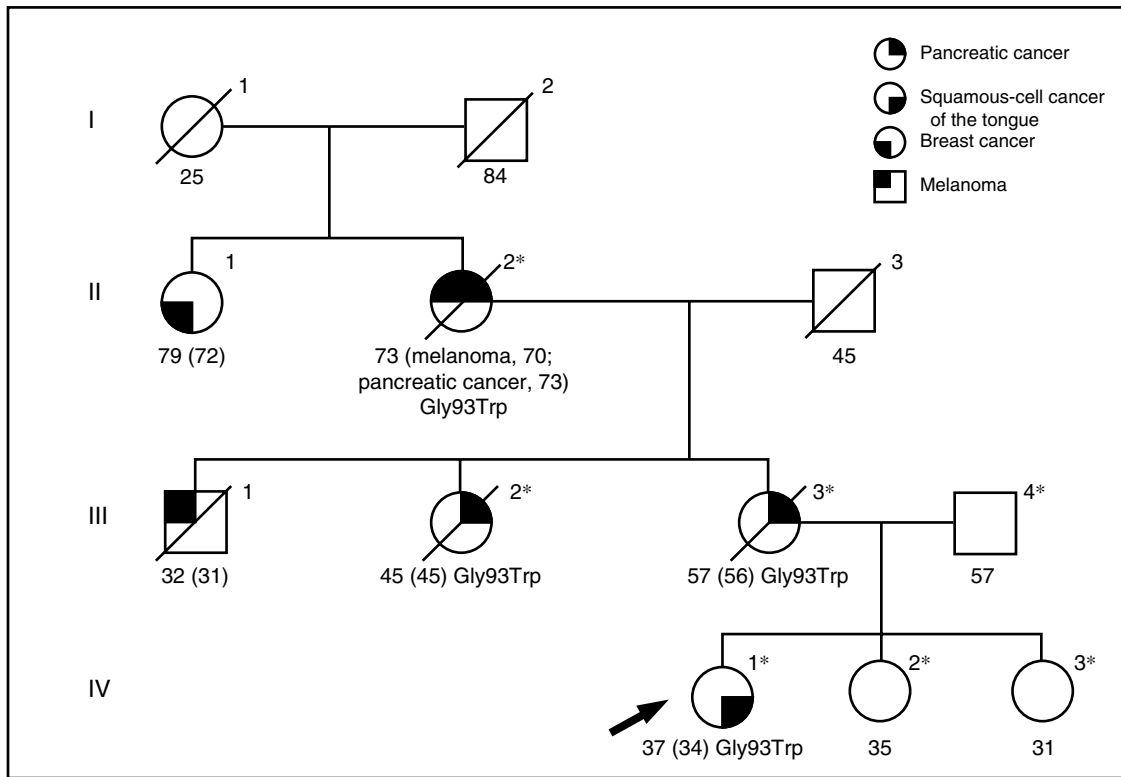


Figure 1. Pedigree of a Kindred with Pancreatic Cancer and Melanoma.

Circles denote female family members, squares male family members, and symbols with a slash deceased family members. The proband is indicated by the arrow. Asterisks indicate family members in whom DNA studies were undertaken. Current age (in years) or age at death is given below each figure, with the age at diagnosis given in parentheses. Family members who are heterozygous for the Gly93Trp mutation are indicated. Subject I-1 died in childbirth.

members. The same Gly93Trp mutation was identified in all affected family members. In each case the mutation coexisted with the normal gene sequence, and the mutation was linked to a specific DNA-marker haplotype common to the affected family members. We believe that the clustering of cancers in this family represents a previously unrecognized familial cancer syndrome that includes pancreatic cancer (perhaps with

an increased incidence of squamous-cell pancreatic carcinoma), melanoma, and squamous-cell carcinoma of the oropharynx. Lynch and colleagues¹² described a single case of pancreatic cancer among the members of five families with malignant melanoma. It is likely that as additional kindreds are identified, the spectrum of associated tumors will change, and more accurate estimates of the lifetime risk of cancer in these families will be possible. Our results emphasize the importance of obtaining careful family histories, particularly for patients who present with multiple cancers or cancer at an early age.

The cosegregation of a *CDKN2* mutation with cancer in this family is further evidence of the role of *CDKN2* in tumorigenesis, particularly in melanoma and pancreatic carcinoma. The Gly93Trp mutation has previously been associated with an increased risk of cancer. The mutation was identified in the constitutional DNA of affected members of three kindreds with familial melanoma.^{10,13} The Gly93Trp variant was also present in 11 family members with melanomas and in 2 with dys-

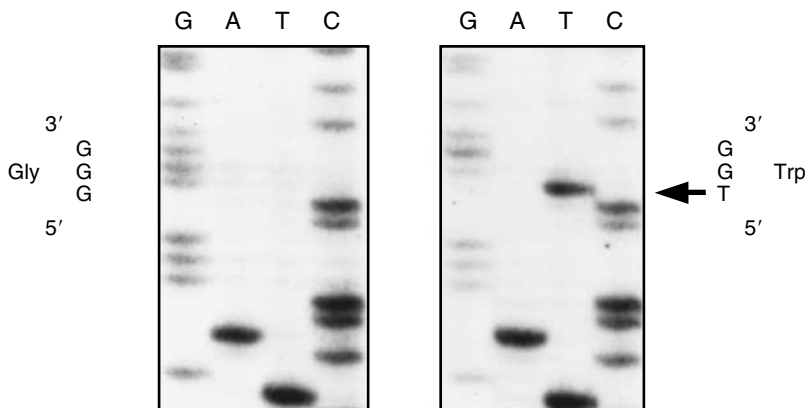


Figure 2. Sequence of *CDKN2* in the Constitutional DNA of the Proband.

The normal sequence at codon 93 — GGG (Gly) — is shown in the left panel, and the sequence that is associated with disease in the kindred — TGG (Trp) — is shown in the right panel. The arrow indicates the G→T transition at codon 93.

plastic nevi. It was not found in 160 unaffected normal subjects, a result that supports the postulate that the Gly93Trp variant is a disease-associated mutation rather than a benign polymorphism. More recently, the Gly93Trp mutation was shown to result in a functionally defective protein that has an impaired ability to inhibit the catalytic activity of complexes of cyclin D1–cyclin-dependent kinase 4 and cyclin D1–cyclin-dependent kinase 6 in vitro.¹⁴

Limited studies of *CDKN2* in pancreatic cancer suggest that the gene also has a role in the pathogenesis of that cancer. In a study of pancreatic adenocarcinoma cell lines and xenografts, *CDKN2* mutations were identified in 79 percent.¹⁵ Recently, somatic mutations of *CDKN2* were found in 37 percent of patients with primary pancreatic adenocarcinomas.¹¹ Families with a high frequency of pancreatic carcinoma have been described,^{16–18} but we are not aware of any other report of a germ-line mutation in such families.

The occurrence of a rare squamous-cell carcinoma of the pancreas in this family and of the squamous-cell carcinoma of the tongue in the proband is intriguing. The frequency of *CDKN2* mutations in a variety of sporadic squamous-cell tumors has varied. A small proportion of head and neck tumors have mutations,¹⁹ whereas *CDKN2* mutations were identified in 51 percent of primary esophageal squamous-cell carcinomas.²⁰ Functional studies of native and mutated forms of the p16 protein point to its participation in arresting the G₁ phase of the cell cycle.^{21,22} This function, together with frequent mutations of the *CDKN2* gene in cancer, suggests the importance of *CDKN2* as a tumor-suppressor gene.

We are indebted to the members of the family described in this report for their willingness to participate in these studies and to Douglas Shevlin, M.D., for his review of the pathological specimens.

REFERENCES

- Malkin D, Li FP, Strong LC, et al. Germ line p53 mutations in a familial syndrome of breast cancer, sarcomas, and other neoplasms. *Science* 1990; 250:1233-8.
- Kinzler KW, Nilbert MC, Su L-K, et al. Identification of FAP locus genes from chromosome 5q21. *Science* 1991;253:661-5.
- Latif F, Tory K, Gnarr J, et al. Identification of the von Hippel-Lindau disease tumor suppressor gene. *Science* 1993;260:1317-20.
- Mulligan LM, Kwok JBJ, Healey CS, et al. Germ-line mutations of the *RET* proto-oncogene in multiple endocrine neoplasia type 2A. *Nature* 1993;363: 458-60.
- Miki Y, Swensen J, Shattuck-Eidens D, et al. A strong candidate for the breast and ovarian cancer susceptibility gene BRCA1. *Science* 1994;266:66-71.
- Cubilla AL, Fitzgerald PJ. Classification of pancreatic cancer (nonendocrine). *Mayo Clin Proc* 1979;54:449-58.
- Kamb A, Gruis NA, Weaver-Feldhaus J, et al. A cell cycle regulator potentially involved in genesis of many tumor types. *Science* 1994;264:436-40.
- Wright DK, Manos MM. Sample preparation from paraffin-embedded tissues. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ, eds. *PCR protocols: a guide to methods and applications*. San Diego, Calif.: Academic Press, 1990:153-8.
- Miller SA, Dykes DD, Polesky HF. A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acids Res* 1988;16: 1215.
- Hussussian CJ, Struwing JP, Goldstein AM, et al. Germline p16 mutations in familial melanoma. *Nat Genet* 1994;8:15-21.
- Bartsch D, Shevlin DW, Tung WS, Kisker O, Wells SA Jr, Goodfellow PJ. Frequent mutations in primary pancreatic adenocarcinomas. *Genes Chromosom Cancer* (in press).
- Lynch HT, Frichot BC, Lynch P, Lynch J, Gurigis HA. Family studies of malignant melanoma and associated cancer. *Surg Gynecol Obstet* 1975;141: 517-22.
- Kamb A, Shattuck-Eidens D, Eeles R, et al. Analysis of the p16 gene (*CDKN2*) as a candidate for the chromosome 9p melanoma susceptibility locus. *Nat Genet* 1994;8:23-6.
- Ranade K, Hussussian CJ, Sikorski RS, et al. Mutations associated with familial melanoma impair p16^{INK4} function. *Nat Genet* 1995;10:114-6.
- Caldas C, Hahn SA, da Costa LT, et al. Frequent somatic mutations and homozygous deletions of the p16 (*MTS1*) gene in pancreatic adenocarcinoma. *Nat Genet* 1994;8:27-32. [Erratum, *Nat Genet* 1994;8:410.]
- Friedman JM, Fialkow PJ. Familial carcinoma of the pancreas. *Clin Genet* 1976;9:463-9.
- Reimer RR, Fraumeni JF Jr, Ozols RF, Bender R. Pancreatic cancer in father and son. *Lancet* 1977;1:911.
- Ehrental D, Haeger L, Griffin T, Compton C. Familial pancreatic adenocarcinoma in three generations: a case report and a review of the literature. *Cancer* 1987;59:1661-4.
- Zhang SY, Klein-Szanto AJ, Sauter ER, et al. Higher frequency of alterations in the p16/*CDKN2* gene in squamous cell carcinoma cell lines than in primary tumors of the head and neck. *Cancer Res* 1994;54:5050-3.
- Mori T, Miura K, Aoki T, Nishihira T, Mori S, Nakamura Y. Frequent somatic mutation of the *MTS1/CDK4I* (multiple tumor suppressor/cyclin-dependent kinase 4 inhibitor) gene in esophageal squamous cell carcinoma. *Cancer Res* 1994;54:3396-7.
- Lukas J, Parry D, Aagaard L, et al. Retinoblastoma-protein-dependent cell-cycle inhibition by the tumour suppressor p16. *Nature* 1995;375:503-6.
- Koh J, Enders GH, Dynlacht BD, Harlow E. Tumour-derived p16 alleles encoding proteins defective in cell-cycle inhibition. *Nature* 1995;375:506-10.

The *Journal's* E-Mail Addresses:

For letters to the Editor:
letters@edit.nejm.org

For information about submitting material for Images in Clinical Medicine:
images@edit.nejm.org

For information about the status of a submitted manuscript:
status@edit.nejm.org