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MUTYH Exon 7 and 13 Mutations Associated with Colorectal Cancer (MAP Syndrome) Are Not Commonly Associated with Sporadic Pancreatic Cancer

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Key Words
MUTYH · *MUTYH*-associated polyposis · Pancreatic cancer · K-*ras* mutations

Abstract
Background: Biallelic *MUTYH* exon 7 and 13 mutations are associated with a high frequency of somatic K-*ras* gene guanine to thymine transversion mutations at codon 12 position 1 in *MUTYH*-associated polyposis patients who have increased risk of colon cancer. The purpose of this study was to determine if a similar association exists between exon 7 and 13 *MUTYH* mutations and pancreatic cancer. ***Methods:*** Genomic DNA samples from 140 patients with pancreatic cancer and 107 controls were sequenced and analyzed for mutations in each of *MUTYH* exons 7 and 13. ***Results:*** Two patients with pancreatic cancer were identified as heterozygous for a *MUTYH* Y165C germline mutation. One pancreatic cancer patient was heterozygous for a G382D mutation and an additional patient was heterozygous for a novel missense mutation, L406M. No biallelic mutations were identified in pancreatic cancer or control subjects. ***Conclusion:*** Despite their association with somatic K-*ras* mutations and an increased risk of colorectal cancer in *MUTYH*-associated polyposis patients, *MUTYH* exon 7 and 13 mutations were not associated with pancreatic cancer in our cohort.

Introduction

Pancreatic cancer is ranked fourth among the causes of cancer deaths in the United States, with an estimated 34,290 cancer-related deaths in 2008 [1]. The prognosis for pancreatic cancer is poor, with overall 5-year survival rates of less than 5% [1]. Despite the recent recognition of premalignant lesions, early spread of tumor cells relative to the onset of symptoms and resistance to both chemotherapy and radiation contribute to the poor prognosis [2]. Due to the low incidence of pancreatic cancer in the general population, screening for this disease should be limited to high-risk individuals. Presently, these high-risk individuals consist of members of pancreatic cancer-prone families in which the probability of developing pancreatic cancer significantly exceeds that predicted for the general population [3]. It has been estimated that up to 10% of pancreatic cancer cases have a family history of pancreatic cancer [4]. Families with germline mutations in *PRSS1* (hereditary pancreatitis), *STK11* (Peutz-Jeghers syndrome), *CDKN2A* (familial atypical multiple mole melanoma), and *BRCA1/2* (hereditary breast/ovarian cancer) are known to be at a greater risk for developing pancreatic cancer; however, the germline mutations conferring the predisposition are unknown for the majority of pancreatic cancer-prone families [4]. Additional genet-

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ic risk factors are likely important and yet to be identified.

Cancer development can often be attributed to the synergistic effect of multiple somatic alterations in oncogenes, tumor suppressor genes, and microRNA genes in addition to germline mutations. The protein product of the oncogene K-*ras* has guanosine triphosphatase activity and is constitutively activated by mutations in codons 12, 13 and 61, resulting in unrestricted cell growth [5]. K-*ras* is activated by mutations in codon 12 in the majority of advanced PanIn lesions and pancreatic cancers [6, 7]. The most prevalent K-*ras* mutations in pancreatic cancer occur at position 2 of codon 12 and are guanine to adenine transitions (GGT → GAT) and guanine to thymine transversions (GGT → GTT) [8].

Guanine to thymine transversions are also common in colorectal adenomas and carcinomas of patients with *MUTYH* (previously known as MYH)-associated polyposis (MAP). MAP is a recently described, autosomal recessive condition similar to familial adenomatous polyposis in which susceptibility to multiple colorectal adenomas and cancer is attributed to germ-line mutations in *MUTYH* and resulting guanine to thymine transversions in the *APC* gene [9, 10]. MUTYH prevents guanine to thymine transversions by removing adenine residues mispaired with guanine and 8-oxo-7,8-dihydro-2′-deoxyguanosine (8-oxoG) [11], a stable derivative of guanine produced by reactive oxygen species during aerobic metabolism [12]. The two *MUTYH* mutations first reported and most frequently observed in MAP are Y165C (A→G at nt 494 in exon 7) and G382D (G→A at nt 1145 in exon 13) [9, 10, 13]. Lipton et al. [9] reported that 64% of MAP cancers and 43% of MAP adenomas tested possessed mutations in codon 12 of K-*ras*. All codon 12 K-*ras* mutations were guanine to thymine transversions at position 1 (GGT → TGT) rather than position 2 as commonly seen in pancreatic cancer. The diminished adenine glycosylase function associated with biallelic *MUTYH* mutations has been identified as responsible for the high frequency of MAP guanine to thymine transversions in *APC* and at position 1 of codon 12 in K-*ras* [9].

Based on the prevalence, nature and location of K-*ras* mutations in MAP, we hypothesized that Y165C and G382D germline *MUTYH* mutations may also contribute to the high frequency of K-*ras* transversions in pancreatic cancer. In this study, we screened for germline *MUTYH* mutations by direct sequencing of *MUTYH* exons 7 and 13 in genomic DNA samples from 140 pancreatic cancer patients.

Materials and Methods

Subject Selection

We identified 140 patients through the Pancreatic Adenocarcinoma Gene Environment Risk (PAGER) study at the University of Pittsburgh diagnosed with pancreatic cancer between 1998 and 2007. We also screened a total of 112 individuals without pancreatic cancer involved in the PAGER and North American Pancreatitis 2 studies at the University of Pittsburgh for *MUTYH* exon 7 and 13 mutations as controls. Among these controls, 2.5% have a family history of pancreatic cancer. All protocols were approved by the University of Pittsburgh Institutional Review Board and informed consent was obtained from all patients and controls prior to study enrollment.

DNA Purification and Polymerase Chain Reaction Amplification of MUTYH *Exons 7 and 13*

DNA was extracted from whole blood using the FlexiGene DNA Kit (Qiagen) buffy coat protocol according to the manufacturer's directions. *MUTYH* exons 7 and 13 in genomic DNA were amplified by polymerase chain reaction (PCR). The primer sequences for exon 7 were 5′-AGGAACGATAGAGGGACTGACG-3′ (forward) and 5′-TACCACCTGATTGGAGTGCAAG-3′ (reverse) and those for exon 13 were 5′-AGGGCAGTGGCATGAGTAAC-3′ (forward) and 5′-AACATCCTTGGCTATTCCGC-3′ (reverse). PCR was completed using 10 ng of genomic DNA, 3.0 mM $MgCl_2$ for exon 7 (2.5 mM for exon 13), 5 μl 10 × PCR buffer II (Applied Biosystems), 25 μM deoxynucleoside triphosphate, 0.1 μM of each primer, 2.5 μl DMSO, and 1.25 U AmpliTaq Gold DNA Polymerase (Applied Biosystems) in a total reaction volume of 50 μl. Thirty cycles of PCR were executed as follows: 94.0°C for 30 s, 54.8°C for 15 s for exon 7 (60.0°C for 15 s for exon 13), 72.0°C for 30 s.

Mutation Screening

MUTYH exons 7 and 13 were screened by direct DNA sequencing in both directions. PCR products were purified in a total reaction volume of 10.05 μl containing 5 μl PCR product, 1 μl 10× rAPid alkaline phosphatase buffer (Roche), 1 U rAPid alkaline phosphatase (Roche), and 1 U *Escherichia coli* exonuclease I (New England BioLabs). Purified PCR products were sequenced using BigDye Terminator Cycle Sequencing Kit (Applied Biosystems) according to the manufacturer's instructions, however with a one eighth dilution, and purified further by ethanol precipitation. Sequences were separated on a 3730xl DNA Analyzer (Applied Biosystems) and analyzed using Sequencher 4.8 software (Gene Codes Corporation).

Results

Subject Demographics

Fifty-six female and 84 male patients with histologically verified adenocarcinoma of the pancreas were tested. The patients had a mean age of 64 years at the time of diagnosis (median 66; range 39–90). The control subjects included 107 individuals without pancreatic cancer with a mean age of 59 years (median 60; range 25–84).