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Genome-Wide Somatic Copy Number Alterations in Low-Grade PanINs and IPMNs from Individuals with a Family History of Pancreatic Cancer

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Abstract

Purpose—Characterizing the earliest chromosomal alterations of pancreatic precursor neoplasms from individuals with a familial aggregation of pancreatic cancer may provide clues as to the loci of pancreatic cancer susceptibility genes.

Experimental Design—We used Illumina 370/660K SNP arrays to conduct genome-wide copy number analysis in 60 benign neoplasms [58 mostly low-grade pancreatic intraepithelial neoplasias (PanIN) and intraductal papillary mucinous neoplasms (IPMN) and two pancreatic neuroendocrine tumors (PNET)] and matched normal tissues from 16 individuals with a family history of pancreatic cancer. PanINs and IPMNs were analyzed for *KRAS* codon 12/13 mutations.

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Disclosure of Potential Conflicts of Interest

J. Leclerc has employment (other than primary affiliation; e.g., consulting) in Lille University Hospital, France, as assistant professor/hospital practitioner. A.P. Klein, R.H. Hruban, and M. Goggins have a licensing agreement with Myriad Genetics for the discovery of *PALB2* as a pancreatic cancer susceptibility gene. No potential conflicts of interest were disclosed by the other authors.

Authors' Contributions

Conception and design: S.-M. Hong, M. Goggins

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Results—Of 40 benign neoplasms with adequate SNP calls and allele ratios, somatic chromosomal copy number changes were identifiable in only nine lesions, including eight of the 38 PanIN/IPMNs (two of which had identical alterations) and one of the two PNETs. Only two precursor lesions had more than one somatic copy number alteration. In contrast, the overwhelming majority (~95%) of PanINs harbored *KRAS* mutations. The chromosomal alterations identified included nine chromosomal arms affected by chromosomal loss and two by chromosomal gain. Copy number loss spanning 9p21.3 was identified in three precursor lesions; two precursors had chromosomal losses affecting 6q and 17p.

Conclusions—Low- and intermediate-grade PanINs and IPMNs from patients with a family history of pancreatic cancer harbor few if any somatic chromosomal alterations. The absence of a locus of recurrent chromosomal loss in most low-grade pancreatic cancer precursor lesions supports the hypothesis that there is no one tumor suppressor gene locus consistently involved in initiating familial pancreatic neoplasia.

Introduction

Almost 10% of patients with pancreatic cancer report a family history of pancreatic cancer (1). Although several pancreatic cancer susceptibility genes have been identified in recent years, the genes responsible for most of the familial clustering of pancreatic cancer is not yet explained. Recently, germ line mutations in *ATM* and *PALB2* have been identified in approximately 1% to 3% pancreatic cancer families through exome analysis (2, 3) expanding the list of known pancreatic cancer susceptibility genes that includes *BRCA2*, *CDKN2A/p16*, *STK11*, and *PRSS1* (4–7). Genome-wide association studies have also identified low-penetrance alleles in *ABO*, *hTERT*, and other loci that have a small average influence on pancreatic cancer risk (8). Environmental risk factors are also likely to contribute to pancreatic cancer risk in affected families (9). Discovering new high-penetrance familial pancreatic cancer susceptibility genes can have rapid clinical impact. Affected family members can undergo gene testing and identifying at-risk carriers enables clinicians and genetic counselors to provide more accurate information about risk and to tailor this information to pancreatic cancer screening. Furthermore, some germ line mutations can impact therapy. For example, biallelic inactivation of *BRCA2*, *PALB2*, and *ATM* renders cells sensitive to PARP inhibitors or radiotherapy (10, 11).

Interestingly, the published reports of subjects with familial pancreatic cancer who have undergone germ line exome analysis reveal that most individuals do not have an obvious candidate pancreatic cancer susceptibility gene mutation (2, 3). The inability to detect susceptibility genes in the majority of individuals who have undergone exome analysis to date suggests that alternative strategies for identifying candidate pancreatic cancer susceptibility genes are needed. We used one such strategy in this study to characterize genetic alterations in the precursor neoplasms in patients with a family history of pancreatic cancer. If there are tumor suppressor loci commonly involved in the early stages of pancreatic neoplastic development, some precursor neoplasms would be expected to have chromosomal alterations at such loci. Most pancreatic ductal adenocarcinomas are thought to evolve through pancreatic intraepithelial neoplasias known as pancreatic intraepithelial neoplasias (PanIN; ref. 12). Patients with a strong family history of pancreatic cancer who have pancreatic lesions identified by pancreatic screening and undergo pancreatic resection often have multifocal PanINs (13). Although most PanINs are not detectable by currently available imaging tests, patients with a familial aggregation of pancreatic cancer often develop another precursor lesion visible by imaging as pancreatic cysts known as the intraductal papillary mucinous neoplasm (IPMN). The ability to detect and survey IPMNs in patients with an inherited susceptibility to pancreatic cancer has led to clinical trials evaluating the role of pancreatic screening in this population (14–19). Many patients who

undergo pancreatic screening harbor both multifocal PanINs and multifocal IPMNs (14–19). Pancreata resected from individuals with a pancreatic cancer and a family history of pancreatic cancers have more PanINs and IPMNs than do pancreata from individuals with sporadic pancreatic cancers (13, 20). The differences in the mean number of precursor lesions in individuals are modest (2.75-fold higher area of precursors in "familial" compared with "sporadic" pancreata; ref. 20), but within the familial population, some familial pancreata have dozens of precursors whereas others have few (13). In addition, more PanIN-3 lesions can be identified in pancreata from those with a family history than those without (20). Overall, the higher overall prevalence of pancreatic precursor lesions and the higher prevalence of high-grade precursor lesions in patients with a familial aggregation of pancreatic cancer supports the hypothesis that many such individuals could harbor germ line mutations in tumor suppressor genes whose loss would accelerate the development and progression of pancreatic precursor lesions.

Genetic alterations common to invasive pancreatic ductal adenocarcinomas such as oncogenic mutations of *KRAS* and telomere shortening are detected in the earliest stages of PanINs and IPMNs (21–26). The small size of PanINs has limited their molecular analysis, and as a result, genetic analyses of PanINs have generally involved a few candidate genes or loci (27, 28). For example, Luttges and colleagues used microsatellite markers to examine the timing of chromosomal losses at 9p, 17p, and 18q in sporadic PanINs (27). The chromosomal loss patterns of familial pancreatic cancers and IPMNs have also been evaluated using micro-satellite markers (28). More recently, high-density single-nucleotide polymorphism (SNP) microarrays have been used to determine genome-wide copy number alterations of invasive pancreatic ductal adenocarcinomas (29), but such analysis has not been applied to sporadic or familial PanINs. In this study, we used Illumina HumanCNV370 and CNV660 genotyping BeadChip arrays to identify chromosomal alterations in whole-genome amplified DNA from low- and intermediate-grade PanINs and IPMNs obtained from patients with a family history of pancreatic cancer to determine whether there are common somatic copy number alterations in these lesions.

Materials and Methods

An overview of our experimental approach is summarized in Fig. 1.

Patients and tissues

Fresh-frozen sections of multiple precursor lesions were obtained from 16 patients with family history of pancreatic cancers who underwent pancreatic resection at the Johns Hopkins Hospital (Baltimore, MD). We selected patients who had precursor lesions identified by histological evaluation of their fresh-frozen resected pancreata and if more than one precursor neoplasm was identified in the resection specimen. After microdissection and copy number analysis, some of these precursor neoplasms failed to yield adequate copy number data. We also limited our analysis to low- and intermediate-grade precursor lesions, rather than high-grade (carcinoma *in situ*) lesions so as to identify the earliest chromosomal alterations that arise during pancreatic neoplasia. One patient carried a germ line mutation in *BRCA1* and the other patient had Peutz–Jeghers syndrome. Apart from germ line *p16* testing (which did not identify any mutations), none of the other patients were tested for germ line mutations in pancreatic cancer susceptibility genes as known genes only explain approximately 10% of the familial clustering of pancreatic cancer. Hematoxylin and eosin-stained frozen section slides were evaluated to assess the degree of dysplasia using criteria described elsewhere (30). The grades of dysplasia of PanINs were classified into PanIN-1, PanIN-2, or PanIN-3 (30, 31). The grade of dysplasia of IPMNs was classified into low-, intermediate-, or high-grade dysplasia following new World Health Organization (WHO) classification (31). There was one incipient IPMN defined as histologically resembling an

IPMN but lacking the size criteria for an IPMN (conventionally ≥ 1 cm; further described elsewhere, refs. 20). Two PNETs approximately 1 cm in size were also analyzed. PNETs were considered to be precursor lesions as they are suspected to have the potential to develop into neuroendocrine cancers. PNETs were included because we have observed some individuals undergoing pancreatic screening to have PNET lesions as well as other precursor lesions, raising the possibility that there could be a common genetic mechanism responsible for their development. Matched normal tissues, including normal duodenum, lymphocytes, spleen, acinar cells, or matched normal pancreatic epithelia, were also collected. Precursor lesions of pancreatic cancers as well as normal pancreatic ductal epithelial cells were meticulously microdissected from frozen sections with the PALM micro-laser system (Carl Zeiss Microimaging Inc.).

DNA extraction, whole-genome amplification, and genotyping

Genomic DNAs were extracted from the micro-dissected samples with QIAamp DNA Micro Kit (Qiagen, Inc.) following the manufacturer's protocol. Extracted DNA was quantified using 3 methods to ensure accurate quantification: QPCR (Quantifiler, Applied Biosystems) and Pico-Green Assay (Invitrogen) and spectrophotometry (NanoDrop).

Whole-genome amplification was conducted using approximately 30 to 40 ng of DNA and the REPLI-g Mini Kit (Qiagen) following the manufacturer's protocol. We also used the Sigma WGA method for comparison in 6 of the same samples. Amplified DNAs were quantified after whole-genome amplification either by Quantifiler or Pico-Green Assay. DNA samples that were sufficiently amplified were included in the SNP array analysis (4 μ g in total or ~ 100 -fold or more amplification) were selected. For most patients, sufficient amounts of normal tissue DNA were available (concentration range, ~ 100 ng/ μ L) for SNP genotyping without the need for whole-genome amplification.

DNA samples were submitted to the Johns Hopkins University Genetics Core for SNP genotyping using the Illumina Human CNV 370 or CNV 660 BeadChip array (Illumina, Inc.). SNP genotyping of the first half of samples was conducted with the 370K array and the second half with the 660K array.

KRAS mutation analysis

The mutation status of *KRAS* codons 12 and 13 in PanINs and IPMNs was determined initially by Sanger sequencing. Then wild-type samples by Sanger sequencing were subjected to high-resolution melt-curve analysis and pyrosequencing. There was insufficient DNA available from several samples for pyrosequencing and melt-curve analysis. Sanger sequencing can detect mutations at high concentrations ($\sim 25\%$ or more), whereas melt-curve analysis and pyrosequencing have detection limits of approximately 5% (32).

Ten nanograms of template DNA was used for each PCR reaction for Sanger sequencing, pyrosequencing, and melt-curve analysis. For each assay, each sample was analyzed in duplicate. For the melt-curve analysis, PCR reactions included 0.1 unit/ μ L of LC Green-Plus dye (Idaho Tech). PCR reactions ended with a final denaturing at 95°C for 30 seconds and 28°C for 30 seconds to generate heteroduplexes. After PCR, plates were analyzed with the Light Scanner mutation analyzer (Idaho Tech) using a melt-curve temperature range of 72°C to 96 °C. Scanning data were analyzed by the Light Scanner software. A fluorescence difference of 5% was set as a cutoff point for identifying variant samples as previously recommended (33).

Pyrosequencing was conducted as described previously with modification (32). Ten nanograms of DNA was PCR amplified with the PyroMark PCR Kit (Qiagen) according to the manufacturer's protocol.

SNP microarray, copy number, and statistical analysis

Raw intensity data were imported from Bead Studio (Illumina) into Partek Genomic Suite (v 4.0, Partek Inc.) for somatic copy number analysis. Regions of copy number alteration were detected with a hidden Markov model (HMM) algorithm in the standard Partek workflow for paired samples. LOH was evaluated by comparing paired normal and precursor lesions (either PanIN or IPMN lesions). The prevalence of alterations was compared by lesion grade and type and associations were examined by Pearson χ^2 test. Statistical analyses were conducted with SPSS version 17. A *P* value <0.05 was considered statistically significant.

Results

A summary of the individuals whose precursor lesions were included in this study is provided in Table 1. Five cases were male and 11 female, and their mean age was 62.4±8.0 years. Five patients had a family history of pancreatic cancer and underwent pancreatic resection for an infiltrating pancreatic ductal adenocarcinoma and the remaining patients underwent pancreatic resection for precursor neoplasms usually after undergoing pancreatic screening because of their family history of pancreatic cancer (Table 1). One patient had Peutz-Jeghers syndrome and another carried a germ line *BRCA1* mutation; none of the other patients were known to carry mutations in pancreatic cancer susceptibility genes. All 16 patients had multifocal PanIN lesions and/or IPMNs in their resection specimen and the proportions of PanINs to IPMNs analyzed reflect the proportions of these lesions in the resected pancreata. Sixty-six precursor lesion samples were submitted for SNP array analysis along with DNA from each case's matched normal tissues. Six of the precursor samples represented experimental replicates to evaluate different whole genome amplification methods. We did not find any significant difference in SNP quality by whole genome amplification method. The remaining 60 samples included 35 PanIN-1 and 12 PanIN-2 lesions, one incipient IPMN, 9 IPMNs with low-grade dysplasia, one IPMN with intermediate-grade dysplasia, and 2 PNETs. The number of precursor lesions in each case ranged from 1 to 13.

Copy number alterations in familial precursor lesions

Although PanIN samples were only included for SNP microarray analysis, if microdissection yielded sufficient DNA for optimal whole-genome amplification (Fig. 1), 8 PanIN samples had SNP call rates insufficient for optimal copy number analysis (call rates, <0.94). An additional 12 samples, (including 6 PanIN-1, 3 PanIN-2, 2 IPMN with low-grade dysplasia, 1 IPMN with intermediate-grade dysplasia) were also excluded, despite optimal SNP call rates (>99%), because their SNP allelic ratios were not sufficiently uniform to rule out somatic copy number alterations. In many samples, the distal ends of several chromosomes showed mild allelic alterations in SNP ratios consistent with the effect of whole-genome amplification (34). Two PanIN lesions from the same individual had an identical chromosomal loss pattern (Table 2, samples 1101 and 1103), despite being obtained from separate regions of the resected pancreas (~3–6 mm apart). Because of their copy number pattern (loss of a ~3 MB of chromosome 9p spanning the *CDKN2A/p16* locus; Fig. 2), we concluded that these 2 samples were from the same PanIN lesion. The remaining 39 precursor lesions included 22 PanIN-1, 7 PanIN-2, 1 incipient IPMN, 7 IPMNs with low-grade dysplasia, and 2 PNETs.

Somatic copy number alterations were observed in 7 of the 37 (19%) evaluable PanIN/IPMNs and 1 of the 2 PNETs. Only 2 precursor lesions (one PanIN-1 and one PanIN-2) had copy number alterations affecting more than one locus. A description of these alterations is provided in Table 2. Figures 2 and 3 depict copy number alterations in representative cases. Among the whole set of precursor lesions, there were 2 loci identified with allelic

imbalances consistent with somatic chromosomal copy number gains and 11 with chromosomal losses. The 2 copy number gains identified involved gain of most or all of a whole chromosome arm and most of the chromosomal losses similarly affected whole chromosomal arms. Three precursor lesions had somatic chromosomal loss involving chromosome 9p, 2 others had loss of 6q, and 2 lesions had 17p loss involving most or the entire chromosomal arm. Because *p16* is a known familial pancreatic cancer susceptibility gene, we examined the germ line DNA from the 3 individuals who had 9p loss in their precursor lesion but did not find any germ line *p16* mutations. Other chromosomal arms affected by somatic copy number alterations in PanINs/IPMNs included 1q, 7p, 10q, 14, 16q, and 19p. While some copy number losses were present in virtually all DNA in the sample (SNP ratios ~2:0; Figs. 2 and 3), other alterations were partial. For example, Fig. 2 contains SNP profiles of a PanIN-2 lesion which has by allele-specific ratios complete loss of portions of 6q, as well as complete loss of all of 17p and part of 17q, and complete gain of most of 16q, but only a portion of the DNA has loss of chromosome 14, suggesting that there is a subclone within the PanIN that has lost chromosome 14. One PNET showed loss of chromosome 11 (Supplementary Fig. S1), consistent with targeting of the *MEN-1* locus. There was a trend toward finding more chromosomal losses in PanIN-1 (14%, 3 of 22 cases) and PanIN-2 (43%, 3 of 7 cases) lesions than in IPMNs with low-grade dysplasia (0 of 7 cases; $P = 0.08$; χ^2 test). The one incipient IPMN was not included in this analysis because it is considered indeterminate with respect to a PanIN versus an IPMN. There was no significant difference in the prevalence of somatic copy number alterations between PanIN-1 and PanIN-2 lesions.

KRAS mutations in familial precursor lesions

Because many of the PanIN and IPMN lesions had no detectable allelic imbalance, we measured their concentrations of mutant *KRAS* to determine their neoplastic purity (Table 3). All precursor lesions were laser capture micro-dissected by an experienced pathologist so as to isolate only neoplastic cells. We found 7 lesions, 4 PanINs and 3 IPMN lesions with *KRAS* mutations by Sanger sequencing did not have any detectable somatic copy number alterations and 3 lesions without detectable mutant *KRAS* by Sanger sequencing that had copy number alterations detected. Other samples had low peaks of mutant *KRAS* by Sanger sequencing. To further determine the concentration of mutant *KRAS* in our precursor lesions, we used melt-curve analysis and pyrosequencing which can quantify lower concentrations of mutant *KRAS* (Table 3; Supplementary Fig. S2). These tests identified mutant *KRAS* in a far higher percentage of familial precursor lesions tested (28 of 30, 93.3%) including 23 of 24 (95.8%) of familial PanIN lesions and 18 of 19 familial PanIN-1 lesions. This prevalence is much higher than had been reported in prior series using less sensitive detection methods (36% of PanIN-1 lesions in one meta-analysis; ref. 35). This high prevalence of mutant *KRAS* in familial PanIN lesions, often in low concentrations, is consistent with our recent findings in sporadic PanINs (25). Somatic copy number alterations were still readily identifiable even in samples with low concentrations of mutant *KRAS* (Figs. 2 and 3, Table 3).

Discussion

Our analysis of genome-wide chromosomal copy number of pancreatic cancer precursor neoplasms from patients with a family history of pancreatic cancer reveals that most low-grade precursor lesions do not harbor any detectable somatic chromosomal copy number alterations. Analysis of these lesions for *KRAS* codon 12/13 mutations revealed that approximately 95% of familial PanIN lesions harbored *KRAS* mutations. These 2 findings, a very high prevalence of mutant *KRAS* lesions and the paucity of somatic chromosomal copy number alterations in early familial precursor lesions, support the hypothesis that many

familial PanINs are not initiated tumor suppressor gene inactivation. Although there are other mechanisms by which germ line tumor suppressor gene mutations can promote tumorigenesis besides the "second-hit" provided by copy number loss (such as haploinsufficiency from a germ line mutation or "second-hit" inactivation by intra-genic mutation or promoter methylation), our results raise the possibility that inherited tumor suppressor gene mutations that predispose to pancreatic cancer usually do so by acting after PanIN initiation and *KRAS* mutation. Our results do not rule out haploinsufficiency or these other potential modes of "second-hit" inactivation. However, genetically targeted tumor suppressor genes known to be inactivated in pancreatic cancers (such as *p16*, *p53*, *SMAD4*, *STK11*, *BRCA2*) are usually affected by biallelic inactivation and loss of alleles (3, 6, 36, 37). We did find a small percentage of PanIN-1 lesions had copy number alteration(s), and in some PanINs, the copy number alteration probably arose before the *KRAS* mutation. For example, PanIN DNA from patient 11 had complete loss of one allele of 9p21, but only 16% of the DNA harbored a *KRAS* mutation which would indicate that the *KRAS* mutation occurred after the 9p loss (Fig. 3). In addition, a PanIN-1 from a patient with Peutz–Jeghers syndrome had 19p copy number alterations at the *STK11* locus, which may have preceded the *KRAS* mutation. However, for the majority of precursor lesions, we find that the oncogenic *KRAS* mutations precede somatic copy number alterations. This observation is consistent with other evidence about pancreatic precursor lesions from individuals with a familial clustering of pancreatic cancer: Their pancreata do harbor more precursor lesions on average than "sporadic" pancreata, but the overall increase in lesion number is modest (20), and only some "familial" pancreata harbor a large excess of precursor lesions more consistent with a germ line gatekeeper gene mutation (13). In contrast, individuals with familial adenomatous polyposis who have germ line mutations in the classic gatekeeper gene, *APC*, develop adenomas that undergo biallelic *APC* inactivation almost as often by copy number loss as by somatic mutation (38–40). The usual precursor phenotype of pancreata from familial pancreatic cancer kindreds appears to be more consistent with that caused by germ line mutations in caretaker genes such as *BRCA2* (41).

The similar high prevalence of *KRAS* mutations in sporadic PanINs and PanINs from those with a family history of pancreatic cancer is also consistent with initial observations about the genetic alterations identified in familial pancreatic cancers; the genes targeted for inactivation are the same as that have been reported for sporadic pancreatic cancers (3, 42). The high prevalence of *KRAS* mutations in "familial" PanINs also highlights the importance of environmental cofactors in the susceptibility of individuals with a familial clustering of pancreatic cancer. And just as we observed for sporadic PanIN-1 lesions which only occasionally have mutations in gene *CDKN2A* and do not have evidence of inactivation of *TP53* or *SMAD4* (26, copy number changes at the loci for *CDKN2A*, *TP53*, and *SMAD4* were detected in only 8.1%, 5.4%, and 0% of our familial precursors, respectively. These genes are usually inactivated in high-grade PanIN-3 lesions (26). It would be useful to formally compare genome-wide copy number profiles of sporadic PanINs but no such study has been reported. In addition, consistent with evidence that *BRCA2* loss occurs late in the pancreatic neoplastic development (41), we did not find any somatic copy number loss at the *BRCA2* locus in our low- and intermediate-grade familial PanINs and IPMNs. In our analysis, the few low-grade precursor lesions that had chromosomal losses often harbored loss at only one locus. Even among precursor lesions that harbored chromosomal alterations, there was no locus consistently affected. The PNETs from individuals with multiple familial precursors, analyzed to identify potential loci altered across the types of precursor lesions, did not have any copy number alterations at novel loci. With the caveats discussed above, these findings raise the possibility that there is no common tumor suppressor locus involved in the development of early familial pancreatic neoplasms. This result is consistent with the results of initial germ line exome analyses of patients with familial pancreatic cancer (43). In the first report, the only deleterious mutations identified among 9 patients with a family

history of pancreatic cancer was one individual who had inactivating mutation in *PALB2*, and germ line mutations in *PALB2* were identified in only approximately 3% of a larger set of pancreatic cancer families (43). Subsequent studies have also found a low prevalence of *PALB2* mutations in pancreatic cancer families (44, 45). In another exome analysis of pancreatic cancer families, truncating *ATM* mutations were found in only 2 of 16 individuals whose exomes were sequenced, and analysis of a large number of families revealed germ line *ATM* mutations in only approximately 2% of cases (2).

This low prevalence of chromosomal alterations in low-grade PanIN and IPMN lesions is in marked contrast to the widespread allelic imbalances observed in pancreatic ductal adenocarcinomas that on average affect approximately 30% of all chromosomal arms (46–48). Because most of these alterations are thought to be present at the onset of invasive cancer, our results suggest that most of the chromosomal aberrations that arise during pancreatic cancer development arise in high-grade lesions (PanIN-3 and IPMNs with high-grade dysplasia).

In addition to chromosomal losses at 9p and 17p, the sites of known tumor suppressor genes, *CDKN2A/p16* and *TP53*, other regions that had chromosomal losses in familial PanINs included 6q, which is a site of frequent chromosomal loss in pancreatic ductal adenocarcinomas (29, 47, 48). Importantly, even after exome sequencing of multiple pancreatic cancer genomes, it is not yet certain whether a tumor suppressor gene is the target of the chromosomal losses at 6q or 19p (3). One PanIN-2 lesion had 17q loss, the site of the recently identified tumor suppressor gene commonly inactivated in cystic neoplasms of the pancreas, *RNF43* (49). *RNF43* is not a common target for mutations in usual pancreatic ductal adenocarcinomas. There were also no novel loci recurrently targeted for chromosomal loss that could provide clues as to the identity of novel pancreatic cancer susceptibility genes.

In summary, we find that most low-grade precursor lesions from patients with a familial aggregation of pancreatic cancer harbor *KRAS* mutations but do not have detectable somatic copy number alterations. The absence of a locus of recurrent chromosomal loss in early precursor lesions associated with familial pancreatic cancer supports the hypothesis that there is no one tumor suppressor gene locus commonly responsible for initiating inherited pancreatic neoplasia.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Translational Relevance

Because of their small size, genome-wide analyses of pancreatic intraepithelial neoplasias (PanIN) have not been reported. Genome-wide copy number analysis has the potential to identify novel tumor suppressor loci. We conducted such analysis of mostly low-grade precursor neoplasms [PanINs and intraductal papillary mucinous neoplasms (IPMN)] from patients with a family history of pancreatic cancer. Our main findings are that more than 80% of these lesions did not have any detectable copy number alterations, whereas approximately 95% of them harbored mutations in *KRAS*. These results support the hypothesis that (i) familial precursor lesions do not begin by tumor suppressor gene inactivation, (ii) *KRAS* mutations may commonly precede tumor suppressor gene inactivation in precursor lesions, and (iii) among precursors that had copy number alterations, there was not one common tumor suppressor locus targeted raising the possibility that there is not one major locus responsible for the majority of familial pancreatic cancer susceptibility.

Figure 1.
Overview of the experimental approach.

Figure 2.

Copy number alterations in case 7. A–D, the top, middle, and bottom are the allele ratio, the copy number, and the allele-specific ratio, respectively. Each red and blue dot in the bottom represent allele, and the dots are displayed on the panel to indicate the ratio of each allele. A–D, PanIN-2. A, chromosome 6 (one allele of most of 6q is absent); B, chromosome 14 (incomplete loss of the chromosome); C, chromosome 16 (gain of most of 16q); and D, chromosome 17 (loss of all of 17p and most of 17q).

Figure 3.

Copy number alterations of chromosome 9 in case 11. A and B, the top, middle, and bottom are the allele ratio, the copy number, and the allele-specific ratio, respectively. Each red and blue dot in the bottom represent allele, and the dots are displayed on the panel to indicate the ratio of each allele. Experimental replicates of case 11 showed identical chromosome 9p21.3 band loss covering *CDKN2A/p16* loci. A, PanIN-2 and B, PanIN-2 sampled from near the lesion in A.

Table 1

Characteristics of cases

Case number	Age, y	Gender	Diagnosis
1	66	Female	Multifocal PanINs (highest, PanIN-3); IPMN, low-grade dysplasia; pancreatic endocrine neoplasm
2	51	Female	2 IPMNs (highest, intermediate dysplasia); multifocal PanINs (highest, PanIN-2; Peutz-Jeghers syndrome)
3	58	Female	2 IPMNs (highest, high-grade dysplasia); 5 PanINs (highest, PanIN-3); pancreatic endocrine neoplasm
4	61	Male	Multifocal PanINs (highest, PanIN-2); 3 IPMNs (highest, low-grade dysplasia)
5	55	Female	Infiltrating pancreatic ductal adenocarcinoma arising from multifocal IPMNs; 4 IPMNs (highest, high-grade dysplasia)
6	75	Male	Infiltrating pancreatic ductal adenocarcinoma; multifocal PanINs (highest, PanIN-3)
7	67	Female	Multifocal PanINs (highest, PanIN-2); pancreatic endocrine neoplasm
8	50	Male	Infiltrating pancreatic ductal adenocarcinoma; multifocal PanINs (highest, PanIN-3)
9	53	Female	Multifocal PanINs (highest, PanIN-3; BRCA1 mutation carrier)
10	68	Female	Infiltrating pancreatic ductal adenocarcinoma; multifocal PanINs (highest, PanIN-3)
11	64	Female	Infiltrating pancreatic ductal adenocarcinoma; multifocal PanINs (highest, PanIN-2)
12	65	Male	IPMN, intermediate-grade dysplasia
14	57	Male	2 IPMNs (highest, low-grade dysplasia)
15	72	Female	2 IPMNs (highest, low-grade dysplasia); multifocal PanINs (highest, PanIN-3)
16	75	Female	Multifocal PanINs (highest, PanIN-3)
18	61	Female	IPMN, intermediate-grade dysplasia; incipient IPMN; multifocal PanINs (highest, PanIN-2)

Table 2

Summary of 370K/660K SNP analysis

Sample number	Patient number	Age, y	Gender	Histology	Chromosome	Start bp	End bp	Aberration	Suspected gene target
106	1	66	Female	PanIN-1	1q	127,385,312	247,249,719	Gain	
107				PanIN-1				No alteration	
108				Neuroendocrine tumor	11	1	134,452,384	Loss	MEN-1
110				PanIN-1				No alteration	
201	2	51	Female	PanIN-1	19p	1	27,886,194	LOH	STK11 ^a
308	3	58	Female	PanIN-1				No alteration	
313				PanIN-1				No alteration	
315				Neuroendocrine tumor				No alteration	
316				Incipient IPMN	9p	809,270	38,035,632	Loss	p16
401	4	61	Male	PanIN-1				No alteration	
413				IPMN with low-grade dysplasia				No alteration	
502	5	59	Male	IPMN with low-grade dysplasia				No alteration	
503				PanIN-1				No alteration	
505				IPMN with low-grade dysplasia				No alteration	
506				IPMN with low-grade dysplasia				No alteration	
507				PanIN-1				No alteration	
508				PanIN-1				No alteration	
509				PanIN-1				No alteration	
601	6	70	Female	PanIN-2				No alteration	
602				PanIN-1				No alteration	
603				PanIN-2				No alteration	
702	7	67	Female	PanIN-2	6q	80,864,173	107,899,991	Loss	
					14	1	106,368,585	deletion#	
					16q	56,563,770	88,827,254	Gain	
					17p	1	22,222,446	Loss	
					17q	23,295,255	78,774,741	Loss	
704				PanIN-1				No alteration	
801	8	50	Male	PanIN-2	17p	1	22,222,446	Loss	

Sample number	Patient number	Age, y	Gender	Histology	Chromosome	Start bp	End bp	Aberration	Suspected gene target
804				PanIN-1				No alteration	
901	9	53	Female	PanIN-1				No alteration	
1001	10	63	Female	PanIN-1				No alteration	
1002				PanIN-1				No alteration	
1101	11	63	Male	PanIN-2	9p	19,900,001	23,210,682	Deletion	p16 ^b
1103				PanIN-2	9p	19,900,001	23,210,682	Deletion	p16 ^b
1203	12	65	Female	IPMN with low-grade dysplasia				No alteration	
1401	14	57	Male	IPMN with low-grade dysplasia				No alteration	
1501	15	72	Female	IPMN with low-grade dysplasia				No alteration	
1601	16	75	Female	PanIN-2				No alteration	
1602				PanIN-1				No alteration	
1603				PanIN-1				No alteration	
1604				PanIN-1				No alteration	
1605				PanIN-2				No alteration	
1606				PanIN-1				No alteration	
1801	18	61	Female	PanIN-1	6q	63,284,396	105,858,243	Deletion ^c	
					6q	110,512,905	170,899,991	Deletion ^c	
					7p	287,385	24,488,091	Deletion ^c	
					9p	7,975,235	13,449,588	Deletion ^c	p16
					10q	92,773,493	104,563,007	Deletion ^c	

NOTE: LOH is assumed to be 19p deletion though allele-specific copy number analysis was indeterminate.

^aPatient is a Peutz-Jeghers syndrome carrier.

^bOn the basis of their genetic pattern, samples 1101 and 1103 were considered to be from the same PanIN.

^cPartial deletion is called when the SNP ratios indicate not all DNA in the sample shows the deletion.

Table 3

Summary of the results of KRAS mutation and LOH analysis

Sample number	Patient number	Age, y	Gender	Histology	KRAS summary	KRAS SS	KRAS HRM	% mutation PS	KRAS PS	Copy number alteration
106	1	66	Female	PanIN-1	G12D	G12D	NT	NT	NT	Present
107				PanIN-1	G12D	WT	Positive	20	G12D	Absent
110				PanIN-1	WT	WT	Negative	0	WT	Absent
201	2	51	Female	PanIN-1	G12D	G12D	NT	NT	NT	Present
308	3	58	Female	PanIN-1	G12V	WT	Positive	27	G12V	Absent
313				PanIN-1	G12V	WT	Positive	7	G12V	Absent
316				Incipient IPMN	Inc	WT	NT	NT	NT	Present
401	4	61	Male	PanIN-1	G12D	WT	Positive	6	G12D	Absent
413				IPMN, low-grade	G12D	G12D	NT	NT	NT	Absent
502	5	59	Male	IPMN, low-grade	G12V	G12V	NT	NT	NT	Absent
503				PanIN-1	G12V	WT	Positive	19	G12V	Absent
505				IPMN, low-grade	G12V	G12V	NT	NT	NT	Absent
506				IPMN, low-grade	WT	WT	Negative	0	WT	Absent
507				PanIN-1	G12V	WT	Positive	27	G12V	Absent
508				PanIN-1	G12D	G12D	NT	NT	NT	Absent
509				PanIN-1	G12V	G12V	NT	NT	NT	Absent
601	6	70	Male	PanIN-2	G12D/G12V	G12D/G12V	NT	NT	NT	Absent
602				PanIN-1	G12D/G12V	G12D/G12V	NT	NT	NT	Absent
603				PanIN-2	G12V	WT	Positive	21	G12V	Absent
702				PanIN-2	G12V	G12V	NT	NT	NT	Present
704				PanIN-1	G12V	WT	Positive	45	G12V	Absent
801	8	50	Male	PanIN-2	G12D	WT	Positive	44	G12D	Present
804				PanIN-1	G12D	WT	Positive	30	G12D	Absent
901	9	53	Female	PanIN-1	Inc	WT	NT	NT	NT	Absent
1001	10	66	Female	PanIN-1	Inc	WT	NT	NT	NT	Absent
1002				PanIN-1	G12V	WT	Positive	7	G12V	Absent
1101	11	63	Male	PanIN-2	G12V	WT	Positive	16	G12V	Present
1103				PanIN-2	G12V	WT	Positive	33	G12V	Present

Sample number	Patient number	Age, y	Gender	Histology	KRAS summary	KRAS SS	KRAS HRM	% mutation PS	KRAS PS	Copy number alteration
1203	12	65	Male	IPMN, low-grade	G12V	WT	Positive	23	G12V	Absent
1401	14	57	Male	IPMN, low-grade	Inc	WT	NT	NT	NT	Absent
1501	15	72	Female	IPMN, low-grade	G12V	WT	Positive	20	G12V	Absent
1601	16	75	Female	PanIN-2	NT	NT	NT	NT	NT	Absent
1602				PanIN-1	G12V	WT	Positive	32	G12V	Absent
1603				PanIN-1	G12V	WT	NT	8	G12V	Absent
1604				PanIN-1	G12V	WT	Positive	25	G12V	Absent
1605				PanIN-2	Inc	WT	NT	NT	NT	Absent
1606				PanIN-1	G12V	WT	Positive	15	G12V	Absent
1801	18	61	Female	PanIN-1	Inc	WT	Positive	NT	NT	Present

NOTE: On the basis of their genetic pattern, samples 1101 and 1103 were considered to be from the same PanIN.

Abbreviations: CN, copy neutral; HRM, high resolution melting curve analysis; Inc, inconclusive; NT, not tested; PS, pyrosequencing; SS, Sanger sequencing; WT, wild-type.