

## Early detection of pancreatic cancer

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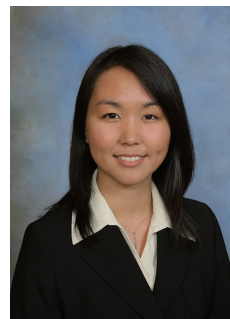
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**Abstract:** Pancreatic adenocarcinoma is a low-incident but highly mortal disease. It accounts for only 3% of estimated new cancer cases each year but is currently the fourth common cause of cancer mortality. By 2030, it is expected to be the 2<sup>nd</sup> leading cause of cancer death. There is a clear need to diagnose and classify pancreatic cancer at earlier stages in order to give patients the best chance at a definitive cure through surgery. Three precursor lesions that distinctly lead to pancreatic adenocarcinoma have been identified, and we have increasing understanding the non-genetic and genetic risk factors for the disease. With increased understanding about the risk factors, the familial patterns, and associated accumulation of genetic mutations involved in pancreatic cancer, we know that there are mutations that occur early in the development of pancreatic cancer and that improved genetic risk-based strategies in screening for pancreatic cancer may be possible and successful at saving or prolonging lives. The remaining challenge is that current standards for diagnosing pancreatic cancer remain too invasive and too costly for widespread screening for pancreatic cancer. Furthermore, the promises of noninvasive methods of detection such as blood, saliva, and stool remain underdeveloped or lack robust testing. However, significant progress has been made, and we are drawing closer to a strategy for the screening and early detection of pancreatic cancer.

**Keywords:** Early detection; screening; pancreatic cancer; familial pancreatic cancer

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## Introduction

Pancreatic adenocarcinoma is a low-incident but highly mortal disease. It accounts for only 3% of estimated new cancer cases each year but is currently the fourth common cause of cancer mortality (1). By 2030, it is expected to be the 2<sup>nd</sup> leading cause of cancer death (2). The overall 5-year survival rate is only 20% for patients with localized disease and a mere 2% for those with distant metastasis (3). This attests to the aggressive biology of the pancreatic adenocarcinoma as well as qualities hindering its early detection. Surgery remains the only possibility for cure for pancreatic cancer; however, only 10-20% of patients are diagnosed with the disease at a stage that is amenable to resection (1). Ultimately, even patients who receive surgery have a 5-year survival ranging between 10-25% (4). There is a clear need to diagnose and classify pancreatic cancer at earlier stages in order to give patients the best chance at a definitive cure. While challenges remain in detecting pancreatic cancer at an early and/or resectable stage, progress is being made.

## Challenges in the early detection of pancreatic cancer

One of the major challenges to detecting pancreatic cancer is its late clinical presentation. By the time pancreatic cancer

is diagnosed, the cancer is usually well-advanced. One study demonstrated that only 7% of pancreatic cancers are considered localized disease at diagnosis. This is startlingly low compared to other malignancies: breast (61%), colon (40%), lung (16%), ovarian (19%), and prostate (91%) (5). Because the pancreas is a retroperitoneal organ, sitting deep in the torso, there are no external lumps that can be palpated or external skin changes that can be seen during an annual routine physical exam, such as may be the case for a breast lesion. Neither is it accessible by a digital exam such as the prostate nor is it readily assessed thoroughly and directly by intraluminal endoscopic videos such as with the colon. Pancreatic cancer often progresses asymptotically and when it does present, its symptoms are non-specific, such as nausea, anorexia, jaundice, and weight loss and abdominal pain (6). Pain, when present, is in the upper abdomen radiating through to the back and is usually associated with a lesion in the body or tail of the pancreas (6). In contrast, tumors of head of the pancreas tend to present with the classic painless jaundice and possibly steatorrhea and early cachexia. Tumors arising from the ampulla of Vater are likely to present initially with jaundice and at an earlier stage, thus increasing their resection potential (6). With the type of blood-work available as the current standard of care for patients with suspected pancreatic cancer, there are no unique patterns of laboratory value that give definitive

diagnosis. Liver function tests may have elevation due to obstruction of bile flow (6). The tumor marker that has been associated with pancreatic cancer is Carbohydrate Antigen 19-9 (CA 19-9), but it lacks sensitivity and specificity, and all the more so in the presence of obstructive jaundice (6). CA 19-9 is approved for monitoring of therapy but not as a diagnostic marker by the FDA (7).

Another barrier in the early detection of pancreatic cancer is its relatively low prevalence. The prevalence of pancreatic cancer in the United States population (all ages) is approximately nine per 100,000, and, if narrowed to individuals above the age of 55 years, it rises to approximately 68 or 100,000 individuals. The positive predictive value of any test improves by increasing the prevalence of the disease being tested for the population being tested (8,9). Because of the low prevalence of pancreatic cancer, the early detection of pancreatic cancer through screening is problematic. For example, if 100,000 people over the age of 55 years were screened for pancreatic cancer using a test with a specificity of 98% and a sensitivity of 100%, it would generate 1,999 false-positive test results but only 68 true-positive results. A specificity of higher than 99% would be required for a more acceptable positive predictive value (9). With a disease like pancreatic cancer that requires invasive and extensive surgery for treatment, such a large rate of false positives cannot be afforded. As of today, there is no method of detection that is fit for screening the general population for pancreatic cancer or its precursor lesions.

### Is early detection possible and beneficial?

Because of the aggressive biology of the disease, the question can be posed about whether early detection of pancreatic cancer is first of all possible and, if possible, whether the mortality of the disease would actually be decreased by subsequent early intervention. Research suggests that there is a window of time where screening of pancreatic cancer would be possible. Iacobuzio-Donahue *et al.* applied a mathematical modeling to the patterns of genetic alternations present in multiple lesions from the same patient and estimated that there is at least ten years during which pancreatic cancer was still in the curative stage, starting from the genetic level of tumorigenesis. Their model, similar to models used by evolutionary biologists, predicted an average of 6.8 years between the birth of the cell serving as the parental clone and the seeding of the index metastasis. It is only in the last 2 years of this decade-long

tumorigenic process that the majority of patients are currently diagnosed with the disease (10). Thus, there is a window of opportunity for the early detection of pancreatic cancer.

Furthermore, the prognosis of patients who receive surgery is better when the tumor is smaller, there is no perineural or lymphovascular invasion, surgical margins are free of disease, and the lesion has a low-grade histology. Tumor differentiation and nodal status also appear to be predictive of mortality (6). The following are results from one of the largest series reported of pancreaticoduodenectomies for pancreatic adenocarcinoma: For cancers <3 cm, median survival was 21 months; 1-, 2-, and 5-year survivals were 73%, 45%, and 23%, respectively. For cancers >3 cm, median survival was 15 months; 1-, 2-, and 5-year survival were 59%, 31%, and 4%, respectively. For cancers with no positive lymph nodes, the median survivals were 23 months; 1-, 2-, and 5-year survivals were 73%, 50%, and 27% respectively. For cancers with positive lymph nodes, the median survival was 17 months; 1-, 2- and 5-year survivals were 63%, 34%, and 16%, respectively. For cancers with negative resection margins, the median survival was 20 months; 1-, 2-, and 5-year survivals were 70%, 43%, and 21%, respectively. For cancers with positive margins, the median survival was 14 months; 1-, 2-, and 5-year survivals were 57%, 26%, and 12%, respectively. For well or moderately differentiated cancers, the median survival was 21 months; 1-, 2-, and 5-year survivals were 72%, 45%, and 22%, respectively. For poorly or undifferentiated cancers, the median survival was 13 months; 1-, 2- and 5-year survivals were 56%, 26%, and 13%, respectively (11). We can assume that the earlier that pancreatic cancer is diagnosed, the more likely the tumor will be less differentiated, smaller, and free from nodal, vascular, or neural involvement. However, formal prospective studies will have to be performed to determine whether screening and early detection will decrease the morbidity and mortality of pancreatic adenocarcinoma. For example, fecal occult blood testing, sigmoidoscopy, and colonoscopy as standard screening for colon cancer were debated until the 1990's when recommending their use as surveillance was finally heralded after numerous studies. Now, screening for colon cancer with colonoscopy is widely accepted and championed (12-14).

### Precursor lesions

Because there are precursor lesions to pancreatic

adenocarcinoma, there is a strong case for effective screening of pancreatic cancer. Three lesions, pancreatic intraepithelial neoplasia (PanINs), intraductal papillary mucinous neoplasms (IPMNs), and mucinous cystic neoplasms (MCNs), have each been identified as distinct precursors to ductal adenocarcinomas of the pancreas. These precursor lesions, along with some small invasive cancers, are curable (9). There is strong evidence that IPMNs and MCNs are present for years before they progress to invasive cancer, which is promising for the purposes of early detection (15-17). PanINs are noninvasive epithelial proliferations within the smaller pancreatic ducts that can be flat or papillary and have a three-tier grading scale: PanIN-1 (low-grade), PanIN-2 (intermediate-grade), or PanIN-3 (high-grade) based on the degree of architectural and cellular atypia (9). Based on the associated mutations with each grade, there appears to be a progression from normal ductal epithelium, to low-grade PanIN, to high-grade PanIN, to localized adenocarcinoma, and to metastatic adenocarcinoma. IPMNs arise in the larger pancreatic ducts and are typically papillary and often produce mucin, as appropriately reflected in their name. IPMNs are larger than PanINs. PanINs are usually <0.5 cm, while most IPMNs are  $\geq 1.0$  cm. IPMNs are more prevalent in the elderly than in the young, and up to a third of IPMNs harbor an associated invasive adenocarcinoma. As is observed with PanINs, there appears to be a progression of genetic alterations correlating to grade, suggesting that IPMNs are a precursor to invasive adenocarcinomas. When an adenocarcinoma arises in association with an IPMN, the IPMN and the invasive carcinoma always have the same mutations. MCNs are large mucin-producing precancerous lesions of the pancreas that almost always arise in the body or tail of the gland and commonly arise in women. They are far less common than IPMNs, accounting for only 16% of resected pancreatic cysts in large surgical series. Similar gene alterations have been reported in MCNs (9). If methods to detect these precursor lesions are perfected, surveillance for early development of pancreatic cancer would be feasible. The next section discusses promising methods of detecting precursor lesions and early-stage pancreatic cancer.

### Advances in detecting early pancreatic cancer

The two broad categories of methods for detecting early-stage pancreatic cancer are (I) imaging and (II) biomarkers. Curable lesions of the pancreas have been

identified and are detectable with technologies that are already a part of clinical practice, including endoscopic ultrasound (EUS), magnetic resonance imaging (MRI), and computed tomography (CT) (9). Molecular-based technologies, such as detecting circulating tumor cells, as well as proteins, mucins, and miRNAs shed by the tumors, are being developed (18-20). If these technologies can be applied effectively in minimally invasive or, ideally, non-invasive bio-samples (e.g., blood, saliva, or stool), this would revolutionize screening for pancreatic cancer. Innovative combinations of the two broad approaches to screening are also being developed, such as molecular-based imaging (18).

### Endoscopic approaches

Contrast-enhanced CT scans is arguably best modality to diagnose a pancreatic tumor, with sensitivity that can be as high as 90% and specificity as high as 99%, and low interobserver variability (21). However, the cost, exposure to ionizing radiation, and risks of intravenous contrast do not make it a feasible screening tool. A screening CT protocol was found to only add 6 additional days of life at an average cost of over \$2,500 per subject (22). MRI has been reported to have similar sensitivity and specificity, but high costs, decreased availability, and required lengthy motionlessness of patients make it a difficult screening tools as well (1,23). Moreover, using whole body CT or MRI as screening tools has the added headache of a high rate of false positive exams. In a recent review of whole body CT imaging for screening, over 90% of subjects were found to have an abnormality, yet only 2% of these findings were clinically important (22).

Of the main modalities used for detecting pancreatic cancer [abdominal ultrasound, EUS, endoscopic retrograde cholangiopancreatography (ERCP), CT, and MRI, and positron emission tomography (PET)], abdominal ultrasound is the least invasive, is a focused exam, does not expose the patient to ionizing radiation (*vs.* CT scans), and is widely available. However, because of its location in the retroperitoneum, the pancreas is not well visualized using standard transcutaneous abdominal ultrasound, thus making it a poor screening tool for the early detection of pancreatic cancer (21). In contrast, EUS has much higher sensitivity when combined with fine needle aspiration (FNA) of tumor tissue. A recent meta-analysis covering the years between 1995 and 2008, the pooled sensitivity and specificity of EUS-FNA were 86.8% and 95.8%, respectively, for

diagnosing a solid pancreatic mass (24-26). ERCP with brush cytology has been replaced by EUS-FNA as the endoscopic test of choice for tissue acquisition due to its higher success rates and decreased risk of post-procedural complications, especially in patients without obstructive jaundice (24). However, EUS-FNA is still an invasive procedure with possibly serious complications and has not yet been demonstrated as a feasible screening tool.

There have been advances such as contrast-enhanced EUS and EUS elastography that may improve the diagnostic accuracy of EUS without the invasive tissue sampling via FNA (25). By administrating micro-bubble agents, the diagnostic accuracy of EUS can be as high as 82% for pancreatic adenocarcinoma (27). EUS elastography, one of the most recent advances in gastrointestinal endoscopy, is a non-invasive technique that measures tissue elasticity in real time using a dedicated probe and system. A number of recent investigations have shown promising results of EUS elastography for diagnosing pancreatic focal lesions (28,29). However, contrast-enhanced EUS and EUS elastography are not widely available techniques and have yet to be tested as screening tools.

The standard method of endoscopic evaluation remains EUS-FNA, and to some degree, ERCP. Because evaluation by direct tumor tissue sampling is invasive, tools are needed to increase the ability to distinguish harmless lesions in the pancreas, such as serous cystadenomas (SCA) from precancerous lesions. Genetic markers have that potential. For example, the mutations present in the neoplastic cells in cystic neoplasms are shed into the cyst fluid and therefore can be detected in cyst fluid (9). Wu and colleagues sequenced the exomes of the four most common cystic neoplasms of the pancreas [SCA, IPMN, MCN, and solid pseudopapillary neoplasms (SPN)] specific genetic patterns that differentiate the four types (30). Goggins and colleagues found that patients with pancreatic ductal adenocarcinoma have significantly higher concentrations of KRAS mutations in their EUS-guided duodenal collections of pancreatic fluid than patients undergoing pancreatic screening and those without evidence of pancreatic disease, but such a test did not reliably distinguish cancer cases from controls. Their work demonstrated the possibility of measuring tumor markers via EUS, but the lasting need for perfecting a method of pancreatic fluid sampling (31). Wu and Goggins' findings suggest that assessment of genetic markers could minimize false positives when detecting pancreatic cancer or precursors via imaging. Patients with precursor lesions could arguably be placed under close

surveillance with stronger assurance that early pancreatic cancer would be detected in a timely manner. Nevertheless, obtaining cyst or pancreatic fluid remains an invasive procedure with risks.

### *Epigenetic biomarkers*

Ideally, pancreatic cancer would be able to be detected at an early stage through minimally or noninvasive methods, such as blood-based screening. One of the promising methods of detecting pancreatic cancer in the serum is methylation-on-beads technology (MOB). MOB is a recently developed nanotechnology that captures and analyzes very small amounts of DNA. Yi *et al.* used this method to detect methylation changes in DNA circulating in 42 serum samples from patients with pancreatic cancer and found a sensitivity of 79% and specificity of 92% for the BNC1 gene promoter and a sensitivity of 48% and specificity of 92% for the ADAMTS1 gene (32). The combination of both markers achieved an overall sensitivity of 81% [95% confidence interval (CI), 69-93%] and specificity of 85% (95% CI, 71-99%) (32). Of note, these two genes had not been particularly associated with pancreatic cancer but for the first time were found to have "dense" methylation in cell lines and in pancreatic cancer and almost no methylation in normal pancreatic samples. Also, these genes did not tend to demonstrate increased methylation in pancreatitis. The rates of methylation increased at every stage of disease, and demonstrated higher rates than those of CA 19-9, except for in stage III and IV where both methylation and CA 19-9 were at 100% (32). Though further research is required to verify and expand these findings, this novel panel represents a highly promising approach for the early diagnosis of pancreatic cancer.

### *Circulating tumor DNA (ctDNA)*

Bettegowda and colleagues also demonstrated nicely how a screening test based on genetic changes, particularly circulating tumor DNA offer great promise. They used digital polymerase chain reaction-based technologies to evaluate the ability of ctDNA to detect tumors in 640 patients with various cancer types, including pancreatic cancer (155 patients). They found that ctDNA was detectable in >75% of patients with advanced pancreatic, ovarian, colorectal, bladder, gastroesophageal, breast, melanoma, hepatocellular, and head and neck cancers, but in less than 50% of primary brain, renal, prostate, or

thyroid cancers. In patients with localized tumors, ctDNA was detected in 73%, 57%, 48%, and 50% of patients with colorectal cancer, gastroesophageal cancer, pancreatic cancer, and breast adenocarcinoma, respectively. ctDNA was often present in patients without detectable circulating tumor cells, suggesting that these two biomarkers are distinct entities (20).

Kinugasa and colleagues recently published a study comparing the K-ras mutations detected by EUS-FNA and in ctDNA from 75 patients with pancreatic cancer. The frequencies of the mutations in tissue samples at G12V, G12D, and G12R in codon 12 were 28 of 75 samples (37.3%), 22 of 75 samples (29.3%), and 6 of 75 samples (8.0%), respectively. The rates of the mutations in ctDNA were 26 of 75 samples (34.6%), 29 of 75 samples (38.6%), and 4 of 75 samples (5.3%), respectively. Overall, the K-ras mutation rates in tissue and ctDNA were 74.7% and 62.6%, respectively, and the concordance rate between them was 58 of 75 samples (77.3%). Survival did not appear to differ by the presence of K-ras mutations in tissue DNA, but the survival of patients with K-ras mutations in ctDNA was significantly shorter than that of patients without mutations in both a development set ( $P=0.006$ ) and an independent validation set ( $P=0.002$ ) (33).

### *miRNAs*

Vicentini and colleagues identified several miRNAs elevated in the sera of patients with resectable pancreatic cancer that had not previously been reported. They particularly found that serum miR-1290 had excellent ability to distinguish serum from patients with low stage pancreatic cancer from control sera and serum miR-1290 levels discriminated patients with pancreatic cancer from controls better than CA 19-9. Interestingly, miR-1290 levels were elevated in the serum of patients with non-invasive IPMNs, raising the possibility that this marker could be used to monitor patients at risk of developing IPMNs (19).

Further study would have to be required but should technology be able to detect miRNA, ctDNA, or epigenetic markers in sera instead of requiring invasive sampling of tumor tissue, cyst or pancreatic fluid, the possibility of blood-work screening for pancreatic cancer in certain populations of patients may be possible.

### *Stool-based modalities*

Besides sera, stool may be the way of non-invasively

screening for pancreatic cancer. Recently, a non-invasive, stool-based screening tool for colorectal cancer has been approved. Cologuard is a kit that patients can use and send via mail for evaluation. This kit includes molecular assays for DNA mutation and methylation biomarkers associated with colorectal neoplasia (KRAS mutations and NDRG4 and BMP3 methylation) and a non-DNA immunochemical assay for human hemoglobin (34). Beta-actin is used as a reference gene to quantify of the total amount of human DNA present in each s included sample (34). The results of assays are given a composite score which is used to determine a positive or negative result. If positive, the patient is to proceed with a colonoscopy.

Cologuard demonstrates that genetic mutations along the digestive tract are detectable in stool. There is yet to be a similar kit for pancreatic cancer, but the advent and widespread approval of Cologuard suggests that such a kit may be possible in the near future.

### *Oral and gut microbiome*

There is an association of the oral microbiome and periodontitis with pancreatic cancer (35). Evidence is limited, but epidemiological data on periodontal disease are worth further investigation. A strong positive association was noted between periodontitis at baseline and subsequent risk of fatal pancreatic cancer ( $RR=1.77$ ) (36). In another study, men with periodontal disease had a 64% higher risk of pancreatic cancer compared to those reporting no periodontal disease (with tooth loss) (37). In yet another study, a 4-fold increase in risk of pancreatic cancer was observed among those with severe periodontitis. This study also showed that elevated antibodies to *P. gingivalis* were associated with a 3-fold increase risk of aerodigestive cancer mortality, though there were not a high enough number of subjects to examine a specific association with pancreatic cancer mortality.

There have also been reports, though inconsistent, of the increased risk of pancreatic cancer in patients with *Helicobacter pylori* (*H. pylori*) (35). One of the original cohort studies to report a positive association for *H. pylori* using data recently updated their analysis with much larger numbers and found no overall or strain-specific associations with pancreatic cancer risk (38,39). A large case-control study reported an effect modification by ABO blood type, where an association between pancreatic cancer risk and CagA-negative *H. pylori* seropositivity was found among individuals with non-O blood type but not among those

with O blood type (OR =2.78; 95% CI, 1.49-5.20; P=0.0014; OR =1.28; 95% CI, 0.62-2.64; P=0.51, respectively) (40). The authors hypothesize that the difference in risk may be explained by differences in terminal binding antigens in gastrointestinal mucins for individuals with non-O blood type (A and B), which influences the binding potential of the *H. pylori*. The same study observed no association between CagA-positive *H. pylori* seropositivity and pancreatic cancer. However, effect modification by blood type was observed in the previous study (39).

### Saliva-based testing

Saliva-based testing is another form of noninvasive testing that may be possible for the early detection of pancreatic cancer. Saliva, like stool, can be easily obtained in most patients, without using a special technique. In a rodent pancreatic cancer model, it was found that exosome-like vesicles carry, drive, and deliver tumor-specific biomarkers into the saliva (41,42). In one study, saliva was used to differentiate between pancreatic cancer patients and patients with normal or chronic pancreatitis using transcriptome profiles in saliva supernatants with a sensitivity of 90% and specificity of 95% (43). Four mRNA biomarkers were combined to discriminate pancreatic cancer cases. A similar approach was used by another group, which assessed metabolites in saliva using mass spectrometry to detect pancreatic cancer-specific signature (44). Another study assessed salivary miRNAs and found that a logistic regression model combining two salivary miRNAs were able to distinguish pancreatic cancer vs. healthy control, pancreatic cancer vs. benign pancreatic tumors, and pancreatic cancer vs. non-cancer (healthy control and benign pancreatic tumor), showing sensitivities of 72.5%, 62.5%, and 70.0% and specificities of 70.0%, 80.0%, and 70.0%, respectively. They concluded that salivary miR-3679-5p and miR-940 possess good discriminatory power to detect resectable pancreatic cancer, with reasonable specificity and sensitivity (45).

### Determining populations to screen

Because of the low prevalence of pancreatic cancer and the imperfect diagnostic methods of imaging and biomarkers, the early detection of pancreatic cancer screening has the greatest chance of being successful when screening is focused on populations that are at a greater risk for pancreatic cancer.

### Screening via clinical risk factors

Based on epidemiological studies, some clinical factors that can be used to narrow down populations for screening are the following: age, hyperglycemia or diabetes, history of chronic pancreatitis or obesity (46-51). A variety of factors increase the risk of pancreatic cancer, but age then family history increases the risk the most. The most common risk factor for pancreatic adenocarcinoma is cigarette smoking (8,52). There is a 2.2-fold increased risk of pancreatic cancer in smokers *vs.* never-smokers (52). 25% of pancreatic cancers are attributable to cigarette smoking and genetic analyses demonstrate that pancreatic cancers from smokers have more mutations than those from never-smokers. It has even been shown that smoking cessation decreases risk, with risk estimates of 1.64 for recent quitters (1-10 years) and 1.12 for long-term quitters (15-20 years ago). Chronic pancreatitis demonstrated a 2.71-fold increased risk of pancreatic cancer. As is the case with diabetes, new-onset pancreatitis can be a sign of a pancreatic neoplasm. The risk of developing pancreatic cancer appears to be highest in rare types of pancreatitis with an early onset, such as hereditary pancreatitis and tropical pancreatitis. Even though there is a strong link between chronic pancreatitis and pancreatic cancer, over a 20-year period only around 5% of patients with chronic pancreatitis will develop pancreatic cancer. Until the development of more sophisticated screening procedures, screening is not recommended for patients with chronic pancreatitis. More reliable screening tests need to be developed to determine the sub-group of patients with chronic pancreatitis at high risk for developing pancreatic cancer. Molecular pathways leading from benign to malignant pancreatic disease need to be better defined. IPMNs, which are often calcified, can resemble chronic pancreatitis. Their role as an explanation for the pancreatitis-pancreatic cancer association needs to be clarified. This may require new epidemiologic studies. There is a need for new epidemiologic studies with a genetic component to explain why such a low percentage of patients with chronic pancreatitis will develop pancreatic cancer. Animal models are required in which pancreatitis is induced using transgenic or gene ablation technology in order to evaluate the factors able to transform chronic pancreatitis in pancreatic cancer (49).

### Familial pancreatic cancer

It is estimated that 5-10% of PDAC has a hereditary basis

with ~80% penetrance (53). Several genetic syndromes are associated with an increased risk of ductal adenocarcinoma of the pancreas, ranging from high-penetrance genes associated with high lifetime risk of pancreatic cancer to low penetrance genes associated with only a slight increase in risk (<1.5-fold). The risk of pancreatic cancer can be quantified if one knows the gene responsible for aggregation of pancreatic cancer in a family. Quantifying risk is important for the design of clinical trials to screen at-risk patients for early curable precancerous lesions (8). Among the familial genetic mutations that have been noted are *BRCA2*, *PALB2*, *BRCA1*, *p16/CDKN2A*, those pertaining to Lynch Syndrome (*bMSH2*, *bMLH1*, *bPMS1*, *bPMS2*, or *bMSH6/GTBP*), those involved in hereditary pancreatitis (*PRSS1* and *SPINK1*), and *STK11*, which is involved in Peutz-Jeghers (8,54-61).

Family registries have helped determine the familial factors that contribute to the risk of pancreatic cancer. Familial pancreatic cancer is defined as at least a pair of first-degree relatives diagnosed with pancreatic cancer in a family (8). The National Familial Pancreas Tumor Registry (NFPTTR) at Johns Hopkins (nfptr.org) have found that families with at least a pair of first-degree relatives diagnosed with pancreatic cancer (known as “familial pancreatic cancer kindreds”) have a 9-fold increased likelihood of developing pancreatic cancer when compared with the general population (62). The risk of pancreatic cancer rose with the number of family members diagnosed with pancreatic cancer such that individuals with three first-degree relatives with pancreatic cancer had a 32-fold increased risk. The team at the NFPTTR has developed a risk-prediction model tool called PancPro for health care providers, allowing for risk assessment to be tailored to each individual’s family history (63). These models can help identify individuals that may have a greatly elevated risk of developing pancreatic cancer in contrast to risk models that predict the risk of pancreatic cancer using low-penetrance single-nucleotide polymorphisms (SNPs) and known pancreatic cancer risk factors (age, diabetes mellitus, heavy alcohol use, body-mass index, and presence or absence of a family history). These latter risk models have not been shown to identify individuals with a significantly elevated risk of pancreatic cancer (64). With the use of high risk models such as PancPro, the positive predictive value of future screening tools, whether imaging, biomarkers or a combination of both can be increased.

## Conclusions

While significant strides have been made, there is still much

work left to be done to make the early detection of pancreatic cancer a reality. Three precursor lesions that distinctly lead to pancreatic adenocarcinoma have been identified, and we have increasing understanding the non-genetic and genetic risk factors for the disease. However, what we do know is still limited and imperfect.

With increased understanding about the risk factors, the familial patterns, and associated accumulation of genetic mutations involved in pancreatic cancer, we know that there are mutations that occur early in the development of pancreatic cancer and that improved genetic risk-based strategies in screening for pancreatic cancer may be possible and successful at saving or prolonging lives. The current standards for diagnosing pancreatic cancer remain too invasive and too costly for widespread screening for pancreatic cancer. Furthermore, the promises of noninvasive methods of detection such as blood, saliva, and stool remain underdeveloped or lack robust testing. There remains a need for cost-effective biomarkers with robust sensitivity and specificity, improved imaging strategies, further research into the risks and benefits of screening, and the identification of high-yield target populations to screen. However, significant progress has been made, and we are drawing closer to a fitting strategy for the screening and early detection of pancreatic cancer.

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## Footnote

*Conflicts of Interest:* The authors have no conflicts of interest to declare.

## References

1. Poruk KE, Firpo MA, Adler DG, et al. Screening for pancreatic cancer: why, how, and who? *Ann Surg* 2013;257:17-26.
2. Rahib L, Smith BD, Aizenberg R, et al. Projecting cancer incidence and deaths to 2030: the unexpected burden of thyroid, liver, and pancreas cancers in the United States. *Cancer Res* 2014;74:2913-21.
3. Compton CC, Mulvihill SJ. Prognostic factors in pancreatic carcinoma. *Surg Oncol Clin N Am* 1997;6:533-54.
4. Distler M, Rückert F, Hunger M, et al. Evaluation of survival in patients after pancreatic head resection for



- ductal adenocarcinoma. *BMC Surg* 2013;13:12.
5. Jemal A, Siegel R, Ward E, et al. Cancer statistics, 2009. *CA Cancer J Clin* 2009;59:225-49.
  6. Badger SA, Brant JL, Jones C, et al. The role of surgery for pancreatic cancer: a 12-year review of patient outcome. *Ulster Med J* 2010;79:70-5.
  7. Oncology: First Test to Monitor Pancreatic Cancer Receives FDA Clearance. *Cancer Network*, 2002.
  8. Wolfgang CL, Herman JM, Laheru DA, et al. Recent progress in pancreatic cancer. *CA Cancer J Clin* 2013;63:318-48.
  9. Lennon AM, Wolfgang CL, Canto MI, et al. The early detection of pancreatic cancer: what will it take to diagnose and treat curable pancreatic neoplasia? *Cancer Res* 2014;74:3381-9.
  10. Yachida S, Jones S, Bozic I, et al. Distant metastasis occurs late during the genetic evolution of pancreatic cancer. *Nature* 2010;467:1114-7.
  11. Winter JM, Cameron JL, Campbell KA, et al. 1423 pancreaticoduodenectomies for pancreatic cancer: A single-institution experience. *J Gastrointest Surg* 2006;10:1199-210; discussion 1210-1.
  12. Winawer S, Fletcher R, Rex D, et al. Colorectal cancer screening and surveillance: clinical guidelines and rationale-Update based on new evidence. *Gastroenterology* 2003;124:544-60.
  13. Walsh JM, Terdiman JP. Colorectal cancer screening: scientific review. *JAMA* 2003;289:1288-96.
  14. Tsai MH, Xirasagar S, Li YJ, et al. Colonoscopy screening among US adults aged 40 or older with a family history of colorectal cancer. *Prev Chronic Dis* 2015;12:E80.
  15. Kang MJ, Jang JY, Kim SJ, et al. Cyst growth rate predicts malignancy in patients with branch duct intraductal papillary mucinous neoplasms. *Clin Gastroenterol Hepatol* 2011;9:87-93.
  16. Wu BU, Sampath K, Berberian CE, et al. Prediction of malignancy in cystic neoplasms of the pancreas: a population-based cohort study. *Am J Gastroenterol* 2014;109:121-9; quiz 130.
  17. Kang MJ, Jang JY, Lee KB, et al. Long-term prospective cohort study of patients undergoing pancreatotomy for intraductal papillary mucinous neoplasm of the pancreas: implications for postoperative surveillance. *Ann Surg* 2014;260:356-63.
  18. Gold DV, Newsome G, Liu D, et al. Mapping PAM4 (clivatuzumab), a monoclonal antibody in clinical trials for early detection and therapy of pancreatic ductal adenocarcinoma, to MUC5AC mucin. *Mol Cancer* 2013;12:143.
  19. Vicentini C, Fassan M, D'Angelo E, et al. Clinical application of microRNA testing in neuroendocrine tumors of the gastrointestinal tract. *Molecules* 2014;19:2458-68.
  20. Bettegowda C, Sausen M, Leary RJ, et al. Detection of circulating tumor DNA in early- and late-stage human malignancies. *Sci Transl Med* 2014;6:224ra24.
  21. Long EE, Van Dam J, Weinstein S, et al. Computed tomography, endoscopic, laparoscopic, and intra-operative sonography for assessing resectability of pancreatic cancer. *Surg Oncol* 2005;14:105-13.
  22. Beinfeld MT, Wittenberg E, Gazelle GS. Cost-effectiveness of whole-body CT screening. *Radiology* 2005;234:415-22.
  23. Sahani DV, Shah ZK, Catalano OA, et al. Radiology of pancreatic adenocarcinoma: current status of imaging. *J Gastroenterol Hepatol* 2008;23:23-33.
  24. Rösch T, Lorenz R, Braig C, et al. Endoscopic ultrasound in pancreatic tumor diagnosis. *Gastrointest Endosc* 1991;37:347-52.
  25. Lee ES, Lee JM. Imaging diagnosis of pancreatic cancer: a state-of-the-art review. *World J Gastroenterol* 2014;20:7864-77.
  26. DeWitt J, Devereaux B, Chriswell M, et al. Comparison of endoscopic ultrasonography and multidetector computed tomography for detecting and staging pancreatic cancer. *Ann Intern Med* 2004;141:753-63.
  27. Fusaroli P, Spada A, Mancino MG, et al. Contrast harmonic echo-endoscopic ultrasound improves accuracy in diagnosis of solid pancreatic masses. *Clin Gastroenterol Hepatol* 2010;8:629-34. e1-2.
  28. Lee TH, Cha SW, Cho YD. EUS elastography: advances in diagnostic EUS of the pancreas. *Korean J Radiol* 2012;13:S12-6.
  29. Giovannini M, Hookey LC, Bories E, et al. Endoscopic ultrasound elastography: the first step towards virtual biopsy? Preliminary results in 49 patients. *Endoscopy* 2006;38:344-8.
  30. Wu J, Jiao Y, Dal Molin M, et al. Whole-exome sequencing of neoplastic cysts of the pancreas reveals recurrent mutations in components of ubiquitin-dependent pathways. *Proc Natl Acad Sci USA* 2011;108:21188-93.
  31. Sadakari Y, Kanda M, Maitani K, et al. Mutant KRAS and GNAS DNA Concentrations in Secretin-Stimulated Pancreatic Fluid Collected from the Pancreatic Duct and the Duodenal Lumen. *Clin Transl Gastroenterol* 2014;5:e62.

32. Yi JM, Guzzetta AA, Bailey VJ, et al. Novel methylation biomarker panel for the early detection of pancreatic cancer. *Clin Cancer Res* 2013;19:6544-55.
33. Kinugasa H, Nouse K, Miyahara K, et al. Detection of K-ras gene mutation by liquid biopsy in patients with pancreatic cancer. *Cancer* 2015. [Epub ahead of print].
34. A stool DNA test (Cologuard) for colorectal cancer screening. *JAMA* 2014;312:2566.
35. Michaud DS, Izard J. Microbiota, oral microbiome, and pancreatic cancer. *Cancer J* 2014;20:203-6.
36. Hujoel PP, Drangsholt M, Spiekerman C, et al. An exploration of the periodontitis-cancer association. *Ann Epidemiol* 2003;13:312-6.
37. Joshipura KJ, Douglass CW, Garcia RI, et al. Validity of a self-reported periodontal disease measure. *J Public Health Dent* 1996;56:205-12.
38. Stolzenberg-Solomon RZ, Blaser MJ, Limburg PJ, et al. Helicobacter pylori seropositivity as a risk factor for pancreatic cancer. *J Natl Cancer Inst* 2001;93:937-41.
39. Yu G, Murphy G, Michel A, et al. Seropositivity to Helicobacter pylori and risk of pancreatic cancer. *Cancer Epidemiol Biomarkers Prev* 2013;22:2416-9.
40. Risch HA, Yu H, Lu L, et al. ABO blood group, Helicobacter pylori seropositivity, and risk of pancreatic cancer: a case-control study. *J Natl Cancer Inst* 2010;102:502-5.
41. Wong DT. Salivary extracellular noncoding RNA: emerging biomarkers for molecular diagnostics. *Clin Ther* 2015;37:540-51.
42. Lau C, Kim Y, Chia D, et al. Role of pancreatic cancer-derived exosomes in salivary biomarker development. *J Biol Chem* 2013;288:26888-97.
43. Zhang L, Farrell JJ, Zhou H, et al. Salivary transcriptomic biomarkers for detection of resectable pancreatic cancer. *Gastroenterology* 2010;138:949-57. e1-7.
44. Sugimoto M, Wong DT, Hirayama A, et al. Capillary electrophoresis mass spectrometry-based saliva metabolomics identified oral, breast and pancreatic cancer-specific profiles. *Metabolomics* 2010;6:78-95.
45. Xie Z, Yin X, Gong B, et al. Salivary microRNAs show potential as a noninvasive biomarker for detecting resectable pancreatic cancer. *Cancer Prev Res (Phila)* 2015;8:165-73.
46. Urayama S. Pancreatic cancer early detection: expanding higher-risk group with clinical and metabolomics parameters. *World J Gastroenterol* 2015;21:1707-17.
47. Anderson KE, Mongin SJ, Sinha R, et al. Pancreatic cancer risk: associations with meat-derived carcinogen intake in the Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial (PLCO) cohort. *Mol Carcinog* 2012;51:128-37.
48. Li D. Diabetes and pancreatic cancer. *Mol Carcinog* 2012;51:64-74.
49. Raimondi S, Lowenfels AB, Morselli-Labate AM, et al. Pancreatic cancer in chronic pancreatitis; aetiology, incidence, and early detection. *Best Pract Res Clin Gastroenterol* 2010;24:349-58.
50. Meng Z, Zhang X, Zheng Q, et al. Acute pancreatitis as an early indicator of pancreatic head carcinoma. *Hepatogastroenterology* 2014;61:1201-6.
51. Li D, Morris JS, Liu J, et al. Body mass index and risk, age of onset, and survival in patients with pancreatic cancer. *JAMA* 2009;301:2553-62.
52. Bosetti C, Lucenteforte E, Silverman DT, et al. Cigarette smoking and pancreatic cancer: an analysis from the International Pancreatic Cancer Case-Control Consortium (Panc4). *Ann Oncol* 2012;23:1880-8.
53. Permuth-Wey J, Egan KM. Family history is a significant risk factor for pancreatic cancer: results from a systematic review and meta-analysis. *Fam Cancer* 2009;8:109-17.
54. Couch FJ, Johnson MR, Rabe KG, et al. The prevalence of BRCA2 mutations in familial pancreatic cancer. *Cancer Epidemiol Biomarkers Prev* 2007;16:342-6.
55. Ferrone CR, Levine DA, Tang LH, et al. BRCA germline mutations in Jewish patients with pancreatic adenocarcinoma. *J Clin Oncol* 2009;27:433-8.
56. Moskaluk CA, Hruban H, Lietman A, et al. Novel germline p16(INK4) allele (Asp145Cys) in a family with multiple pancreatic carcinomas. *Mutations in brief no. 148*. Online. *Hum Mutat* 1998;12:70.
57. Santillan AA, Cherpelis BS, Glass LF, et al. Management of familial melanoma and nonmelanoma skin cancer syndromes. *Surg Oncol Clin N Am* 2009;18:73-98, viii.
58. Wilentz RE, Goggins M, Redston M, et al. Genetic, immunohistochemical, and clinical features of medullary carcinoma of the pancreas: A newly described and characterized entity. *Am J Pathol* 2000;156:1641-51.
59. Whitcomb DC. Genetics of alcoholic and nonalcoholic pancreatitis. *Curr Opin Gastroenterol* 2012;28:501-6.
60. Solomon S, Whitcomb DC. Genetics of pancreatitis: an update for clinicians and genetic counselors. *Curr Gastroenterol Rep* 2012;14:112-7.
61. Korsse SE, Harinck F, van Lier MG, et al. Pancreatic cancer risk in Peutz-Jeghers syndrome patients: a large cohort study and implications for surveillance. *J Med Genet* 2013;50:59-64.

62. Klein AP, Brune KA, Petersen GM, et al. Prospective risk of pancreatic cancer in familial pancreatic cancer kindreds. *Cancer Res* 2004;64:2634-8.
63. Wang W, Chen S, Brune KA, et al. PancPRO: risk assessment for individuals with a family history of pancreatic cancer. *J Clin Oncol* 2007;25:1417-22.
64. Klein AP, Lindström S, Mendelsohn JB, et al. An absolute risk model to identify individuals at elevated risk for pancreatic cancer in the general population. *PLoS One* 2013;8:e72311.

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