



Pierre-Auguste Renoir. *At the Piano*, 1897.

*Guidelines for genetic testing and screening are in early stages of development.*

## Genetics and Prevention of Pancreatic Cancer

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**Background:** *Pancreatic cancer is an aggressive disease with a poor prognosis. Hereditary factors have been reported in up to 10% of cases of pancreatic cancer. The clinical characteristics and genetic abnormalities have been identified for a proportion of this high-risk group, and the development of preventive strategies for these individuals is now a primary goal of cancer clinicians.*

**Methods:** *A review of the current literature regarding the genetics, screening, and prevention of pancreatic cancer and its precursor lesions was undertaken.*

**Results:** *Risk factors for pancreatic cancer include smoking, chronic pancreatitis, and a genetic predisposition. The role of diabetes or a diet high in fat or meat remains unclear. The genetic mutations that accompany pancreatic cancer appear to occur in a temporal sequence, beginning in the earliest of precursor lesions. These mutations are detectable in pancreatic juice and, in conjunction with imaging, form the basis of screening programs for high-risk individuals. Not all precursor lesions will undergo malignant transformation, and testing is currently limited in its ability to determine which lesions will undergo transformation.*

**Conclusions:** *Avoiding tobacco smoking and minimizing risk factors associated with chronic pancreatitis are recommended to reduce the risk of pancreatic cancer. Individuals with a high-risk genetic background require counseling, genetic testing if appropriate (BRCA2 mutation or p16<sup>INK4A</sup> inactivity) and secondary screening for pancreatic cancer in specialist centers. Risk stratification will improve as more genetic abnormalities causing pancreatic cancer are defined.*

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### Introduction

Pancreatic cancer is a leading cause of cancer-related death in the Western world, accounting for 40,000 deaths per year.<sup>1</sup> In the United States, it is the second-leading cause of death attributed to neoplasms of the gastrointestinal tract and is responsible for approximately 30,000 deaths per year.<sup>2</sup> In the United Kingdom, pancreatic cancer is the fifth-leading cause of can-

cer-related death. It is an aggressive disease that is almost uniformly fatal, with the incidence rate approaching the mortality rate. Even with improvements in surgical treatment, the prognosis remains poor, and although knowledge of specific risk factors is advancing, prevention or cure is unlikely. As a result, much focus has been placed on the identification and detection of the specific genetic abnormalities that accompany the disease and their diagnostic and therapeutic applications.

Outside of high-volume specialist centers, surgical resection is associated with a mortality rate of approximately 15% to 30%; within these centers, the rate is now of the order 5%.<sup>3</sup> Without resection, the overall median survival is 4 to 6 months with an estimated 5-year survival rate of 0.4% to 5%<sup>4</sup>; chemotherapy has only a modest effect in improving survival by just a few weeks or months.<sup>5</sup> Resection increases median survival to 13 to 15 months with a 5-year survival rate of approximately 10%, but this may be increased to around 20 months and 24% to 30%, respectively, with adjuvant chemotherapy.<sup>6</sup> Unfortunately, due to the lack of specific symptoms and current limitations in imaging, only 10% to 15% of patients are suitable for a potentially curative resection on presentation.

Tobacco smoking is the most important etiological factor but accounts for no more than 30% of all cases.<sup>7</sup>

The second important risk factor is a familial background, reported in approximately 5% to 10% of cases.<sup>8-10</sup> There may be an association with diabetes<sup>11</sup> and a diet high in fat or meat,<sup>12</sup> although this is unclear. An increased risk is also seen in patients with chronic pancreatitis.<sup>13</sup>

Recent advances in molecular biology have increased our knowledge of the molecular and genetic changes that accompany pancreatic cancer. Coupled with a greater understanding of precursor lesions, it is now possible to hypothesize a progression model for pancreatic cancer akin to the adenoma-carcinoma sequence in colorectal cancer. The recent National Cancer Institute (NCI) "Think Tank" highlighted further understanding of the biology and genetics of pancreatic cancer as a key area of research in order to decrease the mortality and develop preventative measures.<sup>14</sup> Molecular analysis of clinical samples now allows us to detect the genetic mutations associated with pancreatic cancer. This detection, combined with improved risk stratification and the use of other screening modalities, has presented the possibility of detection of the disease at an early or preinvasive stage so that an effective treatment can be offered to high-risk individuals.<sup>15</sup> This article presents an overview of the genetic basis of pancreatic cancer along with a discussion on how this knowledge is used to screen for the disease at an early or preneoplastic stage.

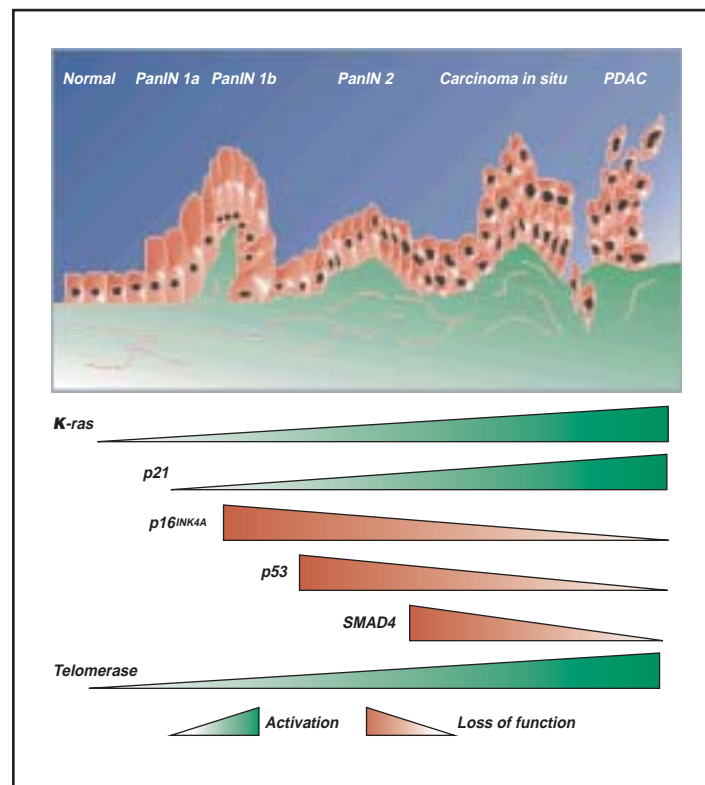


Fig 1. — Pathological and molecular progression model (PDAC = pancreatic ductal adenocarcinoma). Modified from Hruban RH, Goggins M, Parsons J, et al. Progression model of pancreatic cancer. *Clin Cancer Res.* 2000;6:2969-2972. Reprinted with permission by American Association for Cancer Research. Original artwork by Jennifer Parsons Brumbaugh, MA.

## Genetic Overview

Disordered malignant growth resulting from disrupted cell cycle control is accompanied by mutations in several of the genes involved in cell cycle control. These mutations occur in an ordered temporal fashion and correlate closely with histological findings such that a pancreatic cancer progression model is now being developed (Fig 1). Broadly speaking, these genes can be subdivided into two categories: tumor suppressor genes and oncogenes.

### Tumor Suppressor Genes

The cell cycle has several fail-safe mechanisms to ensure the accuracy of each step and maintain the integrity of the genomic DNA. Cyclin-dependent kinases (CDKs), the principal regulators of the cell cycle, are regulated by interaction with a cyclin subunit and can be inactivated by specific inhibitors. A defining point in G<sub>1</sub>, known as the restriction point, marks the position where cells are irreversibly committed to another round of DNA replication. An important regulator of progression through G<sub>1</sub> is the retinoblastoma gene product (pRb). Phosphorylation of pRb (Fig 2), which is a requirement for cell cycle progression, is carried out by the cyclin D and E complexes and results in the liberation of E2F, a transcription factor necessary for the transcription of genes whose products are required for DNA synthesis.<sup>16</sup>

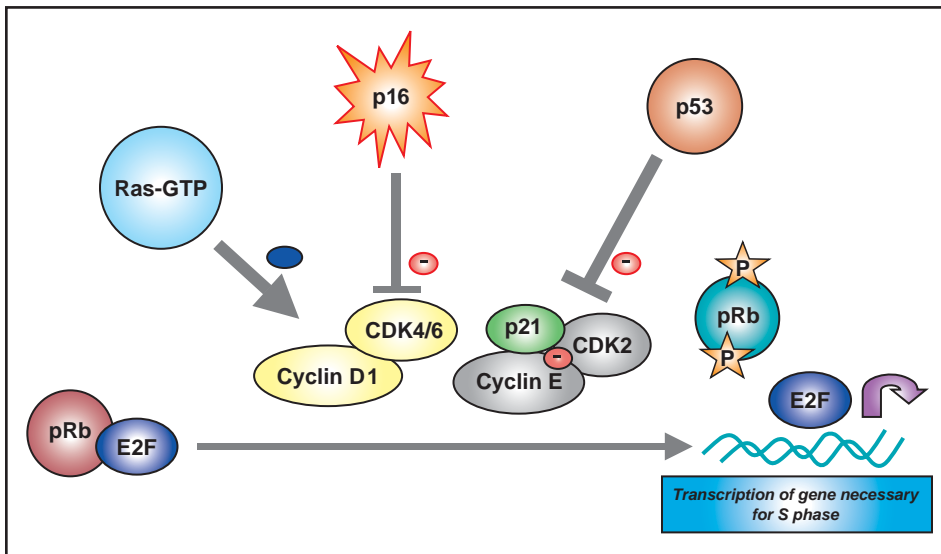


Fig 2. — Schematic representation of the phosphorylation of the retinoblastoma gene product (pRb) that controls passage through G<sub>1</sub> of the cell cycle. Control is exerted by the oncogene K-ras and tumor suppressor genes p53 and p16 through their actions on the cyclin-dependent kinase/cyclin complexes that regulate phosphorylation.

Mutations of tumor suppressor genes, usually both alleles or copies,<sup>17</sup> result in failure of cell cycle regulation. They can be subclassified into gatekeeper genes, caretaker genes, and landscaper genes (Table). Gatekeeper genes (eg, TP53, p16<sup>INK4A</sup>) are mutated early in carcinogenesis and are considered rate-limiting for tumorigenesis. Caretaker genes (eg, BRCA2) maintain genomic integrity, and landscaper genes (eg, PRSS1) act on the tumor microenvironment rather than the tumor itself.<sup>18</sup>

Pancreatic cancer is associated with a high rate of inactivation of three tumor suppressor genes: TP53, p16<sup>INK4A</sup>, and SMAD4. The p53 nuclear protein activates transcription of a cyclin kinase inhibitor p21<sup>WAF1/CIP1</sup>. Following genomic stress, inappropriate growth factor stimulation or expression of oncogenic *ras*<sup>19</sup> increased expression of p53, and thus p21<sup>WAF1/CIP1</sup> resulted in inactivation of specific CDK/cyclin complexes<sup>20</sup> and braking or termination of the cell cycle so that DNA repair or apoptosis can occur. Potential loss of these functions by

inactivation or alteration of the p53 gene have been reported in more than 50% of pancreatic cancers.<sup>21,22</sup>

The p16<sup>INK4A</sup>/p14<sup>ARF</sup> (also known as CDKN2A) locus on chromosome 9q21 encodes these two tumor suppressor genes. Loss of function by mutation, deletion or promoter hypermethylation occurs in 80% to 95% of sporadic cancers.<sup>23</sup> The p16<sup>INK4A</sup> gene regulates cell cycle progression by inhibition of cyclin D/CDK4/6 complexes<sup>24</sup> and thus inhibition of Rb phosphorylation. The interaction of p14<sup>ARF</sup> with MDM2 leads to p53 activation.<sup>25</sup> In pancreatic cancer development, inactivation of p16<sup>INK4A</sup>

seems to be of greater importance than inactivation of p14<sup>ARF</sup> since germline and sporadic mutations have been identified that target p16<sup>INK4A</sup> and leave p14<sup>ARF</sup> intact.<sup>23</sup>

SMAD4 (previously known as DPC4) is deleted or mutated in more than 50% of pancreatic carcinomas.<sup>26</sup> The gene product is an intracellular mediator of the transforming growth factor beta (TGF-β) pathway<sup>27</sup> that inhibits cell growth by inducing G<sub>1</sub> arrest, but it also may have a role in angiogenesis.<sup>28</sup>

### Oncogenes

Derived from protooncogenes, oncogenes encode proteins that act to promote cell growth. Transformation to cancer involves alterations of these genes by point mutations, translocations, or gene amplification resulting in a continuous drive for cell replication. Oncogene-mediated progression within the cell cycle requires only one copy of the protooncogene to be activated by a mutation. However, the presence of a germline null oncogene may reduce the risk of cancer.<sup>29</sup>

*K-ras*, which encodes small guanosine 5'-triphosphate (GTP)-binding cytoplasmic proteins that are involved in signal transduction of growth factors on the cell surface, is the principal oncogene involved in pancreatic cancer and is mutated in approximately 80% of cases<sup>30,31</sup>. Mutations result in trapping the gene product in an activated state and thus leading to uncontrolled cell growth.<sup>32</sup> Recent data have raised the possibility of tumor suppressor properties of *K-ras*. In vivo studies have revealed *K-ras*-mediated inhibition of lung carcinogenesis in mice.<sup>32</sup> A tumor suppressor function of *K-ras* might also exist in the pancreas based on the study of pancreatic cancer cell lines (ASPC-1, CAPAN-1, MIA-PaCa) with loss or underexpression of wild-type *K-ras*.<sup>33</sup> These functions are thought to

Summary of Genetic Mutations in Pancreatic Cancer

Mutation	Reported Incidence of Mutations in Pancreatic Cancer (%)	References
K-ras	53 – 81	30, 31, 50
p16	71, 87	31, 47
p21	75, 85	31, 47
p53	27 – 47	21, 22, 31, 49
SMAD4	50, 85	46, 53
Telomerase	95	38

be produced by the interaction of *K-ras* with proteins involved in cell cycle arrest and apoptosis.<sup>34</sup>

## Chromosomal Instability

Most cancers exhibit complex chromosomal rearrangements. Such genomic instability is thought to be present early in tumorigenesis<sup>35</sup> and is the result of mutations in genes involved in processes such as DNA repair and chromosomal segregation.<sup>36</sup> Each end of a chromosome is composed of a telomere, a special DNA protein structure responsible for maintaining chromosome stability. Telomeres consist of a series of repeat nucleotide sequences that are added by the activity of the enzyme telomerase. Excessive telomeric shortening due to telomerase dysfunction results in complex chromosomal rearrangement and occurs early in tumor development.<sup>37</sup> In cellular immortalization and cancer, elevated levels of telomerase can be detected in clinical samples and have been found to be sensitive in distinguishing benign from malignant disease.<sup>38</sup>

## Pancreatic Cancer Progression Model

A progression model for pancreatic cancer is emerging, and its development is crucial if we are to create screening programs similar to those used successfully in other malignancies. Precursor lesions variously known as metaplasia, hyperplasia, dysplasia, and neoplasia<sup>39,40</sup> have been recognized for more than 45 years.<sup>41</sup> The first link between these lesions and pancreatic cancer was reported by Sommers et al,<sup>41</sup> who noted pancreatic duct hyperplasia in 9% of patients without pancreatic cancer and similar lesions in more than 33% of patients with pancreatic cancer. The study of these suspected precursor lesions has now been aided by the acceptance of a standard classification system and progression model as put forward by the NCI Think Tank.<sup>14</sup> The term PanIN (pancreatic intraepithelial neoplasia) now describes the various changes seen in the pancreatic duct system and is graded 1 to 3 according to the degree of structural dysplasia and cytological atypia. Cell proliferation rates increase with advancing PanIN lesions, consistent with the theory that these are progressive lesions. PanIN-3, previously referred to as carcinoma in situ lesions, demonstrate severe atypia and are likely to progress to invasive carcinoma.<sup>42</sup> Microdissection techniques have revealed mutational profiles in these lesions similar to pancreatic carcinoma, thus providing evidence of a relationship between PanIN and cancer.

The genetic mutations that take place in these precursor lesions appear to occur in a temporal, ordered sequence rather than a random fashion, confirming the theory that some act as gatekeepers in the initiation of neoplastic growth whilst others allow tumor growth and malignant progression. The first to appear is *K-ras*, and it

is thus thought to represent a "gatekeeper" function, although this viewpoint has been challenged.<sup>43</sup> *K-ras* mutations are already present in normal pancreatic ductal cells,<sup>44,45</sup> with the mutation rate increasing up to 100% for PanIN-3 lesions. The p21<sup>WAF1/CIP1</sup> gene has been shown to be overexpressed early in the development of PanIN.<sup>46</sup> This overexpression appears to be cumulative, progressing from 9% in normal ducts to 85% in invasive carcinoma. Its exact role, however, remains unknown. Loss of p16<sup>INK4A</sup> may be the next hit, occurring slightly later than *K-ras*. Allelic loss has been detected in PanIN-1,<sup>47,48</sup> and inactivation has been found to increase up to 100% in invasive cancers.<sup>47</sup> Overexpression of p53 is a later event still, usually occurring in PanIN-2 and -3.<sup>47,49-51</sup> Loss of SMAD4 protein expression, occurring in less than 50% of PanIN-3 lesions,<sup>28,52</sup> is predominately seen in invasive cancers and may signify a poorer prognosis.<sup>53</sup>

Independent of PanIN grade, telomere shortening occurs in 96% of PanIN lesions, with a higher frequency than other genetic abnormalities.<sup>54</sup> It may be this first event that leads to the acquisition of the chromosomal abnormalities that ultimately result in invasive cancer.

## Genetic Risk Factors in Pancreatic Cancer

Up to 5% to 10% of pancreatic cancer cases are due to a primary genetic factor<sup>8-10</sup>; in certain families, this is associated with an autosomal dominant pattern of inheritance.<sup>10</sup> The genetic risk factors associated with pancreatic cancer can be divided into three groups: (1) defined genetic syndrome associated with pancreatic cancer, (2) familial clusters of pancreatic cancer with no obvious genetic syndrome, and (3) pancreatic cancer in primary relatives of patients with a nonpancreatic cancer.

### Familial Pancreatic Cancer

Familial pancreatic cancer (FPC) was first described in 1987.<sup>55</sup> The causative mutation remains unknown, although recent work has identified a subset of patients with BRCA2 germline mutation.<sup>56</sup> A recent study by Eberle et al<sup>57</sup> of a single large kindred suggests that there is a linked gene on 4q32-q34, and they are currently pursuing candidate genes in this large region. The European Registry of Hereditary Pancreatic Diseases (EUROPAC) uses the following criteria to identify patients at increased risk: an individual with two or more first-degree relatives with pancreatic cancer, at least three relatives of any degree with pancreatic cancer, or any two relatives with pancreatic cancer if the sum of their ages at diagnosis was less than 110 years.

### BRCA2 Mutations

The BRCA2 protein product plays a diverse role through its interaction with proteins involved in cell cycle regula-

tion, transcriptional regulation, and DNA repair.<sup>58</sup> Loss of function is thought to lead to chromosomal instability, and carriers of the defective gene have a 26% to 86% increased risk of developing breast cancer.<sup>59,60</sup> The penetrance for pancreatic cancer appears to be lower;<sup>61</sup> in recent studies, germline BRCA2 mutations (mostly 6174delT) were found in approximately 5% of patients with pancreatic cancer who had no family history of pancreatic cancer and in up to 17% in FPC families. In all but two cases, however, the affected individuals were Ashkenazi Jews.<sup>61-63</sup> Thus there was uncertainty as to the general relevance of these results since the carrier frequency of the 6174delT mutation in the general Ashkenazi population is approximately 1%.<sup>64</sup> More recently, in a joint German-EUROPAC study, 5 (19%) of 26 FPC families were found to harbor significant BRCA2 germ-line mutations and none had Jewish ancestry.<sup>56</sup> These findings suggest that BRCA2 testing may be appropriate in pancreatic cancer screening.

### **Familial Atypical Multiple Mole-Melanoma Syndrome**

The familial atypical multiple mole-melanoma (FAMMM) syndrome is an autosomal dominant-inherited syndrome with incomplete penetrance. It is characterized by multiple atypical nevi, familial clustering of malignant melanoma, and an increased incidence of extracutaneous cancers. Its pathogenesis has been linked to inactivation of the p16<sup>INK4A</sup> tumor suppresser gene,<sup>65</sup> and carriers have a 2-fold increased risk of pancreatic cancer.<sup>66</sup>

### **Hereditary Pancreatitis**

Hereditary pancreatitis is an autosomal dominant condition characterized by recurrent childhood attacks of acute pancreatitis resulting in the development of chronic pancreatitis in teenage years. Any form of pancreatitis is thought to pose a risk for pancreatic cancer development,<sup>13</sup> ranging from a 15- to 25-fold risk in sporadic chronic pancreatitis<sup>67-69</sup> to a 70- to 100-fold risk in hereditary pancreatitis.<sup>13,70</sup> It is believed that an imbalance in proteases and their inhibitors resulting from genetic mutations leads to autodigestion and inflammation of the pancreas. Two of the key mutations in hereditary pancreatitis are in the PRSS1 gene, resulting in intracellular accumulation of trypsin and autodigestion,<sup>71</sup> and the gene coding for the synthesis of a serine protease inhibitor, Kazal type 1 (SPINK1).<sup>72</sup>

### **Peutz-Jeghers Syndrome**

Peutz-Jeghers syndrome consists of multiple oromucosal and intestinal hamartomas. It is associated with the development of cancer at multiple sites and has an autosomal dominant pattern of inheritance with a relative risk of pancreatic cancer estimated at 132.<sup>73</sup> The responsible genetic abnormality is mutation of the LKB1/STK11 gene that encodes for serine/threonine kinase with an as yet undefined role.<sup>74</sup>

### **Hereditary Nonpolyposis Colorectal Carcinoma**

Affected individuals of this autosomal dominant condition have an increased risk of colonic and extra-colonic cancers. Caused by mutations in the DNA repair genes HMSH2, HMLH1 and HPMS2 the proportion of familial cancers associated with these mutations appears to be less than p16<sup>INK4A</sup> and BRCA2, but the exact risk of pancreatic cancer is unknown.<sup>75</sup>

### **Ataxia Telangiectasia**

Ataxia telangiectasia is an autosomal recessive condition that is associated with the loss of the ATM (ataxia telangiectasia mutated) gene. The protein product has an important role in the cellular response to genetic stress. Although the mechanisms are unclear, ATM induces cell cycle arrest and DNA repair or apoptosis via p53 and p53-independent pathways.<sup>76</sup> Carriers of the mutated gene have an approximately 3-fold relative risk of pancreatic cancer.<sup>77</sup>

### **Li-Fraumeni Syndrome**

Germline mutations in p53 have been described as Li-Fraumeni syndrome an autosomal dominantly inherited condition, which predisposes to several neoplasms. Pancreatic neoplasms however are rare<sup>78</sup> and the exact risk is unknown due to limited data availability.

### **Familial Adenomatous Polyposis**

Characterized by the appearance of hundreds to thousands of colonic polyps that invariably undergo malignant transformation, familial adenomatous polyposis is an autosomal dominant condition with near complete penetrance. There is an increased risk of extra-colonic malignancy and approximately a 4.5-fold increased risk of pancreatic cancer.<sup>79</sup>

## **Prevention and Screening Strategies**

### **Lifestyle**

Key prevention strategies focus on the elimination of direct environmental risk factors (eg, tobacco smoking) and indirect factors that promote chronic pancreatitis, principally excess alcohol consumption. Approximately 70% of cases of chronic pancreatitis are attributable to alcohol,<sup>80</sup> but overall the contribution to all pancreatic cancers is less than 1%. Currently, the role of a high-fat and/or meat diet remains debatable.<sup>12</sup>

### **Predictive Diagnosis**

Predictive diagnosis by genetic analysis permits the identification of germline mutations known to be associated with the disease. This does not necessarily equate with the certainty of pancreatic cancer development (there are no reliable data with regard to the

degree of penetrance) or with the likely time when the cancer will arise if this should be the case. Given the prevalence of the disease and the limited sensitivity and specificity of current screening tests, primary screening for preinvasive disease is of potential use only in high-risk patients. BRCA2 mutational testing in FPC<sup>56,62</sup> and p16<sup>INK4A</sup> testing in patients with FAMMM<sup>81</sup> have shown the most promising results to date but have yet to reach widespread clinical application. Of these, BRCA2 testing is slightly more robust in that patients in known BRCA2 mutation families who test negative do not require secondary screening. At present, it is not clear whether the same can be said in p16<sup>INK4A</sup> families. Patients considered to be high risk should be offered participation in screening programs. However, given the limited clinical experience of these genetic tests and the professional counseling required, this should be performed in a specialist research environment.<sup>82</sup> Multicenter registries such as the EUROPAC are essential. Individuals who test positive should undergo secondary screening to detect the cancer at a potentially preneoplastic stage, which may be up to a year before a neoplasm is clinically apparent.<sup>15,46,83</sup> If genetic analysis is suggestive of dysplasia and there is evidence to support this, then it would be reasonable to proceed to total pancreatectomy<sup>84</sup> since the multifocal nature of these dysplastic lesions in high-risk groups precludes any form of pancreas-preserving procedure. Indeed, reports now suggest that high-risk individuals benefit from intensive screening.<sup>15</sup>

### **Screening Using Molecular Markers**

The most extensively investigated genetic alteration is mutation of the *K-ras* oncogene, an early and probably essential event in the pathogenesis of pancreatic cancer.<sup>85</sup> It is possible to detect specific *K-ras* mutations in the pancreatic juice,<sup>86</sup> fine-needle aspirates,<sup>87</sup> duodenal fluid,<sup>88</sup> bile,<sup>89</sup> and stool samples<sup>90,91</sup> of patients with pancreatic cancer. However, this can be affected by both the mode of collection and the assay method.<sup>86</sup> Although *K-ras* mutations can be detected in patients with chronic pancreatitis, limiting its sensitivity and specificity as a primary screening test,<sup>92</sup> they may indicate a subgroup of patients who require further long-term follow-up.<sup>93</sup> Serum screening for *K-ras* mutations in combination with measurement of the tumor marker CA19-9, a cell surface glycoprotein, has been reported to improve the sensitivity of *K-ras*,<sup>94</sup> and it may be that the future role for *K-ras* is as an adjunct to other genetic tests until more sensitive screening tests are developed.

Detection of p53 mutations appears to be more promising, with a greater specificity for pancreatic cancer, even in cases with chronic pancreatitis. In conjunction with mutant *K-ras* analysis, p53 detection in both stool and pancreatic juice offers enhanced detection of

pancreatic cancer.<sup>90</sup> Yamaguchi et al<sup>95</sup> detected genetic abnormalities from pancreatic juice in 92% of patients suffering with pancreatic cancer, but only 26 patients were included in the study and controls were lacking. Moreover, p53 mutations appear to occur relatively late in the molecular pathogenesis of pancreatic cancer and may therefore limit its use in detecting early lesions.

Both p16<sup>INK4A</sup> and SMAD4 deletions have been detected in pancreatic juice, but at this time, they do not appear to confer any additional diagnostic power in the detection of early pancreatic cancer.<sup>96</sup> More recent work has shown that detection of methylation status of p16<sup>INK4A</sup> and to a lesser degree p14<sup>ARF</sup> in pancreatic juice can significantly discriminate benign from malignant disease. Using a methylation-specific polymerase chain reaction protocol for p16<sup>INK4A</sup> methylation, Klump et al<sup>97</sup> demonstrated a 100% specificity for malignant disease. Similar results were obtained by House et al<sup>98</sup> who found gene promoter methylation in 55% of patients with invasive intraductal papillary mucinous tumors compared to only 20% in non-invasive tumors.

Telomerase activity that is highly specific for malignancy can be detected in small cellular samples such as pancreatic juice and bile<sup>99</sup> as well as in fine-needle aspirates.<sup>100</sup> Iwao et al<sup>101</sup> reported the detection of telomerase activity in patients with pancreatic cancer but not chronic pancreatitis, and there are anecdotal reports suggesting that telomerase activity can be detected in clinically silent early disease.<sup>102</sup> It also may develop as a diagnostic tool in pancreatic cancer since telomerase activity appears to be an accurate screening marker for the early diagnosis of cervical cancer.<sup>103</sup>

High throughput technologies such as serial analysis of gene expression (SAGE) and microarray technology have empowered genetic analysis by allowing the analysis of hundreds and thousands of genes at a time and thus making these technologies useful in screening programs. Several studies have revealed overexpression of a number of genes in pancreatic cancer, each with the potential to become a biomarker.<sup>104</sup> Analysis of the protein content of a cell or tissue offers a more functional analysis and is capable of detecting posttranslational modifications that are often present only in malignancy. The combination of proteomics with techniques such as microdissection, which overcomes the inherent problem of tissue heterogeneity in such complex samples, offers new and exciting opportunities in the search for novel biomarkers and therapeutic targets.<sup>105</sup>

### **Secondary Screening**

If molecular analysis of either serum or pancreatic juice reveals a mutation, secondary screening is advised using tests such as endoscopic luminal ultrasound (EUS) and multislice computed tomography (CT). A more aggressive screening approach such as endoscopic retrograde cholangiopancreatography (ERCP) may

be used if the initial diagnostic workup proves to be negative, although its use in this setting is controversial. The exact modality of choice should be governed by an individual institution's experience.<sup>84</sup>

Both CT and magnetic resonance imaging (MRI) can be used to image the pancreas. However, they are limited by parenchymal pathology secondary to diseases such as chronic pancreatitis, and this precludes their use as screening investigations. During endoscopic examination of the upper gastrointestinal tract, the pancreatic duct may be cannulated and the pancreatic juice sampled or contrast injected to provide fluoroscopic images of the pancreatic duct (ERCP). ERCP can detect subtle changes in the pancreatic ducts. Currently, pancreatic juice is probably the most suitable sample for the genetic analysis of early pancreatic cancer. However, ERCP has a 5% to 10% complication rate, and these effects can sometimes be severe.

Alternatively, an intraluminal ultrasound may be used to obtain detailed images of the pancreas and also the parenchymal tissue (EUS). Inflammation and fibrosis are common in the parenchyma in conditions such as pancreatitis; however, the parenchyma is often normal in other cancer conditions such as FPC or Peutz-Jeghers syndrome. Brentnall et al<sup>15</sup> reported their experience of screening high-risk individuals from three families with FPC. Seven pancreatectomies were done after screening by EUS revealed abnormalities in 10 of 14 individuals. All seven patients had high-grade dysplasia on histological examination. No data were available regarding the timing of screening, although the authors advise 10 years prior to the earliest pancreatic cancer in an affected pedigree. The general applicability of these results is uncertain due to the bias in selecting individuals to undergo EUS. Both ERCP and EUS may have a role in screening examinations; however, in the presence of background pathology, the power of these modalities to identify early pancreatic neoplasia remains to be established.

## Conclusions

Key recommendations in lowering risk of pancreatic cancer include avoiding tobacco and excessive alcohol consumption and adopting a healthy lifestyle. Although FPC is rare, patients with suspected FPC need counseling and should be considered for germline BRCA2 mutation analysis as a prelude to secondary screening. Others who should undergo secondary screening include those with hereditary pancreatitis and Peutz-Jeghers syndrome. Screening of these individuals is justifiable both scientifically and economically.<sup>106</sup> However, given the limited data available, screening should be done within a research environment and patients should be enrolled on a patient registry.<sup>82</sup> Furthermore, the exact penetrance associated with these

genetic abnormalities has not been established; many healthy individuals may have pancreatic dysplasia that will never progress to neoplasia.<sup>107</sup>

While providing a framework for cancer progression, the progression model does not imply that all low-grade PanIN lesions will progress to infiltrating cancers. Therefore, additional studies are required to determine whether specific morphological or genetic features can predict which PanIN lesions will progress. At present, there is no clear correlation between EUS and dysplasia. Furthermore, the phenotype of FPC is not well described, implying that it may not have the same appearances or presentation as sporadic cancer. In the future, risk stratification will improve with the identification of more genetic alterations responsible for developing an increased risk to pancreatic cancer and with advances in radiological techniques.

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