Hereditary pancreatitis and secondary screening for early pancreatic cancer

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Abstract

Hereditary pancreatitis is an autosomal dominant disease with incomplete penetrance (80%) [1-10], accounting for approximately 1% of all cases of pancreatitis. It is characterized by the onset of recurrent attacks of acute pancreatitis in childhood and frequent progression to chronic pancreatitis [11-13]. Whitcomb et al. identified the cationic trypsinogen gene (PRSS1) on chromosome 7q35 as the site of the mutation that causes hereditary pancreatitis [14]. The European registry of hereditary pancreatitis and familial pancreatic cancer (EUROPAC) aims to identify and make provisions for those affected by hereditary pancreatitis and familial pancreatic cancer. The most common mutations in hereditary pancreatitis are R122H, N29I and A16V but many families have been described with clinically defined hereditary pancreatitis where there is no PRSS1 mutation [1].

It is known that the cumulative lifetime risk (to age 70 years) of pancreatic cancer is 40% in individuals with hereditary pancreatitis [15]. This subset of individuals form an ideal group for the development of a screening programme aimed at detecting pancreatic cancer at an early stage in an attempt to improve the presently poor long-term survival. Current screening strategies involve multimodality imaging (computed tomography, endoluminal ultrasound) and endoscopic retrograde cholangiopancreatography for pancreatic juice collection followed by molecular analysis of the DNA extracted from the juice. The potential benefit of screening (curative resection) must be balanced against the associated morbidity and mortality of surgery. Philosophically, the individual's best interest must be sought in light of the latest advances in medicine and science following discussions with a multidisciplinary team in specialist pancreatic centres.

Key words: hereditary pancreatitis, cationic trypsinogen gene (PRSS1), R122H, N29I, A16V, EUROPAC, pancreatic cancer, secondary screening, Ca19-9, CT, EUS, ERCP, K-ras, p53, p16.

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Hereditary pancreatitis

Hereditary pancreatitis was first described by Comfort et al. in 1952 when working at the Mayo clinic [16]. Comfort et al. described a family with four definite and two suspected cases of relapsing chronic pancreatitis in childhood/adolescence. The observed pattern of inheritance appeared to follow an autosomal dominant mode but with an incomplete penetrance. This observation has since been confirmed by other groups in Europe and North America [2-10]. Indeed, the incomplete penetrance has been noted by various groups with a figure of 80% penetrance being widely accepted [1]. However, as affected individuals are more likely to be tested for the mutation than unaffected individuals and families with few affected members are less likely to be recruited, estimates of penetrance may be overestimated.

Hereditary pancreatitis accounts for approximately 1% of all cases of pancreatitis. It is characterized by the onset of recurrent attacks of acute pancreatitis in childhood and frequent progression to chronic pancreatitis [11-13]. The classic clinical and demographic characteristics include recurrent episodes of pancreatitis during childhood, equal gender distribution, the frequent presence of pancreatic duct stones, a positive family history, and the absence of other known causes of pancreatitis [13,17,18]. The EUROPAC definition of hereditary pancreatitis is two or more first-degree relatives, or three or more second-

degree relatives, in two or more generations with recurrent acute pancreatitis and/or chronic pancreatitis in the absence of other precipitating or causative factors such as gallstones, tropical pancreatitis or excess ethanol consumption [1].

The aetiology of hereditary pancreatitis remained obscure for almost 50 years since first described by Comfort et al. in 1952 [16], until the application of modern molecular genetic techniques. Linkage analysis using microsatellite markers, established cosegregation between the disease phenotype and the long arm of chromosome 7 [19-21]. Once the hereditary pancreatitis gene was mapped to 7q35, positional cloning using a candidate gene approach was employed, whereby genes already known to be in that region were sequenced. Soon afterwards Whitcomb et al. identified the third exon of the protease serine 1 or cationic trypsinogen gene (PRSS1) on chromosome 7q35 as the site of the mutation that causes hereditary pancreatitis [14]. The PRSS1 protein contains 247 amino acids with an eight amino acid activation peptide and a 15 amino acid signal sequence [22].

The European Registry of Hereditary Pancreatitis and Familial Pancreatic Cancer

The European Registry of Hereditary Pancreatitis and Familial Pancreatic Cancer (EUROPAC) was established in 1997 following the realization by a group of European pancreatologists of the need to identify and make provisions for individuals and families affected by inherited diseases of the pancreas, specifically hereditary pancreatitis and familial pancreatic cancer.

The EUROPAC study (www.liv.ac.uk/surgery/europac.html) was established as a European collaboration and in 2002, a formal collaboration was established with a similar research based registry, the Nationale Fallsammlung Familiäres Pankreaskarzinom (FaPaCa or the German National Case Collection of Familial Pancreatic Cancer) of Marburg, Germany (www.med.uni-marburg.de/e-einrichtungen/fapaca/).

The aims of the EUROPAC study have evolved with advances identified in published scientific research and with the identification of new areas of key interest in hereditary pancreatitis and familial pancreatic cancer.

The aims of the study include:

(I) to study and establish the phenotypic and genotypic relations with respect to hereditary pancreatitis (HP) and familial pancreatic cancer (FPC),

(II) to stratify the risk to family members of developing cancer and other clinical manifestations of the inherited condition,

(III) to identify pancreatic cancer susceptibility genes,

(IV) to develop a robust, evidence based secondary screening programme for the detection of early pancreatic cancer in these high risk groups with emphasis on the development and identification of molecular based techniques and markers,

(V) to provide a support service for individuals, their families and for physicians through a comprehensive, multidisciplinary network system of specialists including pancreatologists (surgeons and gastroenterologists), clinical geneticists, and other affected individuals across Europe,

(VI) to provide recommendations, with an accredited molecular genetics service on germline gene testing for genetic mutations that might predispose an individual to pancreatic cancer or pancreatitis,

(VII) to collaborate with other research groups across Europe and beyond in advancing pancreatic research through the exchange of data and materials through both national and international meetings and to publish high quality data in journals that will impact clinical practice across the globe in these disease groups.

Mutations in the cationic trypsinogen gene and variants

Since identification of PRSS1 as a disease gene, a number of different mutations have been identified. The two most frequently occurring mutations in HP are R122H and N29I. These two mutations have been identified in families with hereditary pancreatitis from Europe [23-27], Asia [28] and the Americas [14,29,30].

R122H

The R122H mutation is a single guanine (G) to adenine (A) transition mutation in the third exon of PRSS1 that results in an arginine (CGC) to histidine (CAC) missense substitution at amino acid residue 122. Note that originally residue 122 was referred to as position 117 according to the consensus position with chymotrypsinogen; hence the mutation was referred to as R117H.

The trypsin molecule contains a calcium binding pocket near the side chain connecting the two globular domains of the molecule. This side chain (the autolysis loop) contains amino acid position 122, which is a target for attack by other trypsin molecules. Enzymatic cleavage of the side chain at arginine 122 (R122) by the second trypsin leads to rapid destruction of the first trypsin molecule (autolysis). The autolysis loop is flexible and R122 may come near to the calcium binding pocket. As the concentration of soluble calcium rises, calcium enters the calcium binding pocket and limits exposure of R122 to enzymatic attack by another trypsin [31]. It is widely assumed, with some biochemical support [32-35], that the substitution of histidine for arginine results in a reduction in the destruction of autoactivated trypsinogen in a calcium dependent fashion.

The R122H mutation was easily identified as it created a novel recognition site for the restriction endonuclease AflIII. However, Howes et al. demonstrated that a neutral polymorphism within this enzyme recognition site may produce a false negative result [36]. An alternative mutation specific polymerase chain reaction approach was therefore developed for detection of the mutation even in the presence of the polymorphism [36].

N29I

A second mutation in PRSS1 was subsequently discovered a year later in two affected families without the R122H mutation [37]. A single adenine (A) to thymine (T) transversion mutation (N29I) was identified in exon 2 which results in a change from asparagine (AAC) to isoleucine (ATC) at amino

acid 29, the mutation was previously known as N21I according to the chymotrypsinogen consensus numeration.

The mechanism accounting for how N29I causes pancreatitis is uncertain, although in light of the assumed mechanism of action of R122H and the clinical similarities between R122H and N29I phenotypes, it was suggested that the mechanism must involve increased trypsin activity [37]. This may be due to enhanced autoactivation of trypsinogen, alteration of the binding of pancreatic secretory trypsin inhibitor (PSTI/SPINK1) or impairment of trypsin inactivation by altering the accessibility of the initial hydrolysis site to trypsin. Whitcomb et al. predicted conformational changes in the crystallographic structure of trypsin [38] which could explain a reduced accessibility to the calcium binding pocket. An alternative model was proposed by Nishimori et al., who suggested that the N29I mutation alters the native structure of the PRSS1 gene to a sheet structure [28]. It was implied that this conformational alteration might impair trypsin activation. Sahin-Toth and collaborators used direct biochemical approaches to investigate the mechanism rather than structural modelling and concluded that the N29I mutation increased autoactivation under acidic conditions [33]. This is the most widely accepted mechanism at the time of writing and contrasts with the perceived model for R122H pathology (i.e., reduced inactivation following autoactivation). Despite the apparently significant difference between the pathological mechanisms of N29I and R122H, initial reports from the EUROPAC registry indicate a remarkably similar pathophysiology of the disease in patients with the two mutations [1].

A16V

A third mutation where there is a cytosine (C) to thymine (T) missense mutation has been identified in exon 2 that leads to an alanine (GCC) to valine (GTC) substitution at codon 16 (A16V) [27]. This mutation affects the first amino acid of the trypsinogen molecule and thus directly the cleavage site for the signal peptide. The mechanism by which pancreatitis is initiated remains speculative, but given the position of the mutation at the edge of the signal peptide it is widely believed to involve defects in secretion.

The A16V mutation was identified during a study to determine the spectrum and frequency of mutations in the PRSS1 gene in 44 children/adolescents with chronic pancreatitis [23]. Thirty of these individuals were found to have idiopathic pancreatitis and fourteen hereditary pancreatitis. R122H was identified in one individual; A16V was found in three individuals with presumed idiopathic pancreatitis and in one said to have hereditary pancreatitis. The A16V mutation was also identified in seven first-degree relatives of these patients but only one had clinically apparent pancreatitis, suggesting low penetrance of this mutation.

Variants

The main mutations (N29I and R122H) have exclusively been found in patients with hereditary pancreatitis and, although A16V mutations were originally identified in patients with no clear family history, this mutation has not yet been identified in individuals with ethanol-induced or tropical pancreatitis [1,39-43]. In addition to these three principle mutations, there are multiple variants of the PRSS1 gene as detailed in a recent review by Howes et al. [1]. These include: -28delTCC (a three base pair deletion 28 base pairs upstream from start codon) [25], D19A [44], D22G [45], K23R [25], N29T [46], P36R [40], Y37X [47], G83E [40], K92N [40], L104P [39], R116C [39,48], V123M [40] and C139F [39]. All these variants are rare and in some cases the link with inherited pancreatitis is only suggestive. Two neutral polymorphisms (D162D [39] and N246N [39]) have also been described.

Other disease genes

Although mutations in the Kazal type 1 serine protease inhibitor (SPINK1/PSTI trypsin inhibitor) [49-58] and cystic fibrosis transmembrane conductance regulator (CFTR) [52, 59-62] genes have been associated with cases of pancreatitis of various aetiology, no other gene apart from PRSS1 has been shown to have mutations that cause hereditary pancreatitis. However, many families have been described with clinically defined hereditary pancreatitis where there is no PRSS1 mutation [1]. This indicates that there is at least one more disease gene left to be identified.

Presentation of hereditary pancreatitis

It is crucial to note that data on individuals and their families with hereditary pancreatitis, such as that collated by the EUROPAC study group are hierarchical in structure on account of the nesting of affected individuals within their families and thus they are not completely independent [1]. Howes et al. demonstrated the variation distributed within a family and between families by way of multi-level modelling [1]. This paper was the first that was large enough to use hierarchical statistical analyses in studying the relationship between biological and demographic factors of individuals with hereditary pancreatitis [1].

Howes et al. found that their cohort of patients (n=418 affected) presented with symptoms of pancreatitis at an early age, with a median onset of symptoms at 12 years (95% Confidence Intervals /CI/: 10,13), with over 70% of individuals developing symptomatic pancreatitis by the age of 20 years [1]. Lowenfels et al. looked at a large cohort of individuals with hereditary pancreatitis (n=412 affected) from 16 countries and found the mean age of symptom onset to be 14.1 years with an equal sex ratio but with a slightly more common paternal inheritance pattern (57%) [63].

Howes et al. demonstrated that individuals with R122H mutations presented 10 years earlier (95% CI: 8,12) in comparison to those individuals with the N29I mutation or compared with individuals with no PRSS1 mutation, who had a median age of presentation of 14 years (95% CI: 11,18) and 14.5 years (95% CI: 10,21), respectively [1], although not reported in the paper the data set also showed no evidence for any preference for paternal transmission (unpublished observation).

These findings of early disease onset are fairly consistent with the published literature [1,6-8,13,27,28,37,63-68] reporting significant earlier symptom onset in R122H mutation carriers. Interestingly, Keim et al. studied 101 individuals and failed to

demonstrate any significant difference in age of symptom onset between R122H and N29I mutation carriers [6]. This may be accountable to the small study number and the hierarchical structure of individuals nested within families. Bias arises given that members of families are similar in contrast to random selection of individuals from a population.

Amann et al. provides one of the few studies looking at identical twins in hereditary pancreatitis [10]. They found that the median age of symptom onset of hereditary pancreatitis in concordant twins was almost identical, with similar ages of onset seen in matched siblings and a significantly different age of symptom onset from individuals from age-, sex-, and mutation-matched controls.

Such observations suggest an important role for genetic background, aside from the causative mutations, in determining disease progression. However, bias is probable as siblings are likely to share a common environment as well as a common genetic profile.

Symptoms of hereditary pancreatitis

Howes et al. of the EUROPAC Study group have provided the largest detailed study of hereditary pancreatitis to date [1]. At the time of their guillotine, 527 individuals had been recruited from 14 countries of which 418 individuals from 112 families were affected. This was 58 (52%) families of whom 222 individuals (53%) were characterised by R122H mutations, 24 (21%) of families had N29I mutations (94 individuals, 22%) and 21 (19%) of families had no PRSS1 mutation (72 patients, 17%). Howes et al. demonstrated an overall median of 1.88 attacks (interquartile range: 0.63-3.0) per year, which was unrelated to the type of PRSS1 gene mutation or gender (multi-level modelling) [1]. Not all of the symptomatic episodes of pancreatitis were severe enough to warrant hospital admission, with the median number of admissions to hospital for complications of pancreatitis being 0.3 (interquartile range: 0.08-1.0); other attacks were managed at home or by their General Practitioner (personal physician) [1,69]. The number of hospital admissions was unaffected by gender, however, individuals with PRSS1 mutations did have a tendency for fewer hospital admissions than those with no identified causative mutation. This reached significance when comparing patients with the N29I mutation and those who did not carry a PRSS1 mutation [1]. Approximately, 90% (158/176) of individuals reported that symptomatic episodes lasted no more than one week; the remaining 10% (18/176) had attacks over one week. The duration of acute pancreatitis was not influenced by either gender or PRSS1 mutation status [1]. Prior to the Howes et al. study [1], Gorry et al. reported on two large families with PRSS1 mutations [37]. They found that 86% (24/28) of individuals in the R122H family (n=28) had more than five hospital admissions in contrast to the N29I family (n=15) where there were just 47% (7/15). In a larger study by Keim et al. the clinical characteristics of 30 families with hereditary pancreatitis consisting of six families with the N29I mutation (n=25) and 21 families with the R122H mutation (n=76) were examined [6]. In the N29I group, 24% (6/25) had no symptoms or atypical symptoms and 40% (10/25) mild symptoms. In the R122H group, 26% (20/76) had no symptoms or atypical symptoms and 42% (32/76) mild symptoms. Keim et al. admit that their sample size was small and that the clinical scoring system to classify chronic pancreatitis was not validated [6].

Secondary screening for early pancreatic cancer in high risk groups

It is estimated that 5-10% of pancreatic cancers are attributable to genetic factors [70,71]. Bartsch identified three clinical settings where there may be an inherited predisposition to pancreatic cancer [72]. Firstly, as an adjunct to a familial cancer syndrome associated with an increased risk of pancreatic cancer, as in familial atypical multiple mole melanoma (FAMMM) syndrome [73] and Peutz-Jeghers syndrome [74]. Secondly, as an inherited predisposition to pancreatic cancer linked to another condition; genetic disorders known to predispose to cancer of the pancreas include: hereditary pancreatitis [15,16] and cystic fibrosis [75]. Finally, there are a group of families with apparent autosomal dominant inheritance and a predisposition for pancreatic cancer with no known causative gene (familial pancreatic cancer) [70]. For the purposes of this article, only hereditary pancreatitis will be dealt with.

Hereditary pancreatitis and pancreatic cancer risk

Lowenfels et al. on behalf of the International Hereditary Pancreatitis Study Group estimated that the cumulative lifetime risk (to the age of 70 years) of cancer of the pancreas to be 40% in patients with hereditary pancreatitis [15]. This was supported by Howes et al. in a larger study [1] (see *Fig. 1*, *Tab. 1* and *2*). Lowenfels et al. also reported that paternal transmission of hereditary pancreatitis was associated with a much greater lifetime risk of developing pancreatic cancer [15] but the EUROPAC study group showed that there was no significant difference between paternal and maternal transmission [76].

In cancer syndromes where the gene is unknown it is not clear which individuals are at risk as many family members will not be gene carriers. This is not an issue with hereditary pancreatitis as it is likely that the pancreatic cancer in these families relates to the pancreatitis rather than directly from the gene mutation, therefore only individuals with pancreatitis would be screened.

The justification for secondary screening

Pancreatic cancer is an aggressive disease with a poor prognosis, representing 2% of all new cases of cancer but leading to 5% of all cancer deaths [77]. The median survival is approximately 4-6 months with only 5-10% of individuals being candidates for a surgical resection [78].

The prevalence of pancreatic cancer in the general population (8-12 per 100,000) is too low even in high-prevalence areas such as Northern Europe and North America to permit screen-

Figure 1. **Time to pancreatic cancer showing no significant differences by mutation status. (Reprinted form "Clinical and Genetic Characteristic of Hereditary Pancreatitis in Europe" by Howes et al. with permission from the American Gastroenterological Association** [1])

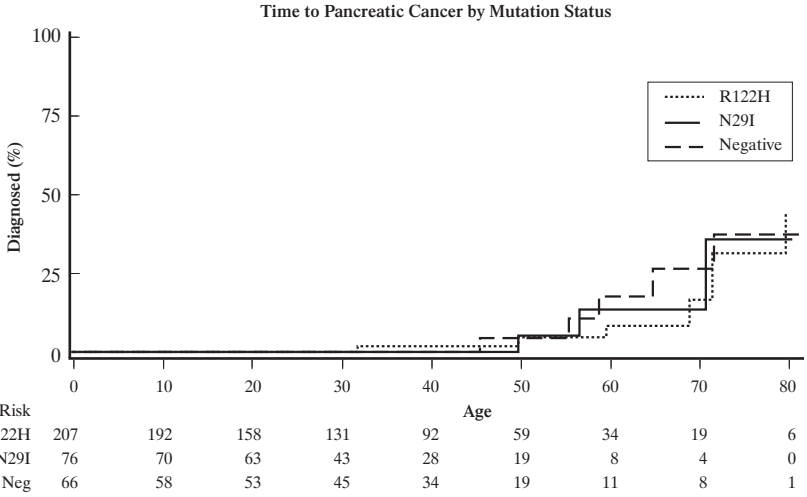

Table 1. **Cumulative risk for the development of pancreatic cancer in hereditary pancreatitis in the EUROPAC Study (n=375)** [1]

Age of risk (Years)	Cumulative Risk of Pancreatic Cancer (%)	95% Confidence Intervals
40	0.5	0.0-1.3
50	3.4	0.4-6.5
60	9.8	3.6-16.0
70	18.8	8.6-29.0
80	33.3	19.0-47.5

Table 2. **Estimates for the development of pancreatic cancer in three large studies of hereditary pancreatitis**

Study	Lifetime Risk	Number of Cancer Cases	One Cancer per Person Years
Lowenfels et al. [15]	40%	8	1066
Keim et al. [6]	-	3	1200
Howes et al. [1]	33%	26	703

ing of the asymptomatic population, given the diagnostic accuracy of present detection methods [79]. However, in the case of hereditary pancreatitis secondary screening can be justified – the primary screen would be to identify the family and the individual with pancreatitis. The secondary screen would attempt to identify those patients with an early asymptomatic cancer which was amenable to curative surgical resection. The diagnostic tests used should provide a high positive predictive value to avoid missing any surgically resectable cancers and a high negative predictive value to prevent unnecessary surgery.

The greatest concern when carrying out screening is the harm that could be caused to individuals with no malignancy. This could result from unnecessary surgery, although with hereditary pancreatitis this would involve resection of a diseased rather than a healthy pancreas. Harm may also be inflicted on a patient directly as a result of the screening modality, but this concern is reduced if the modality is applied as part of the normal management of pancreatitis. The presence of pancreatitis is an indication for screening, but distinguishing a pancreas with a small tumour from a diseased pancreas is more difficult than distinguishing a small pancreatic tumour in an otherwise healthy organ.

Imaging modalities such as endoluminal ultrasound scanning (EUS), and endoscopic retrograde cholangiopancreatography (ERCP) have been employed to distinguish patients with pancreatic cancer from patients with symptoms routinely mistaken for pancreatic cancer, such as pancreatitis [80]. The EUROPAC study group also employs molecular screening of pancreatic juice obtained at ERCP as adjuncts to imaging modalities to stratify risk, reducing the frequency of screening in lower risk patients and increasing the positive predictive value of the imaging [81].

Management of high risk individuals

A screening programme can only be justified if a positive result will offer some possibility of treatment; primary screening, by classifying individuals as high risk for pancreatic cancer is therefore, controversial. Arguments can be made that lifestyle changes may reduce risk and that advice on prevention including the avoidance of smoking are therefore, beneficial. Smoking has been suggested to increase risk of cancer in hereditary pancreatitis [82]. On the other hand, there is the issue of increased anxiety for the family unit and lack of clear evidence that such lifestyle changes will overcome the genetic risk [81]. Thus, having identified individuals at high risk, there is an ethical requirement to offer enrolment on a secondary screening programme, which would allow tumours to be identified at a treatable stage.

Guidelines were established during the third international

symposium on inherited diseases of the pancreas in Milan in 2001 for the secondary screening of patients with hereditary pancreatitis. These included patients being given the opportunity to discuss the variability in the penetrance of the pancreatic susceptibility gene(s) with a clinical geneticist, who would also address issues of psychological stress, insurance and employment discrimination [83].

The strategy of secondary screening is based on the assumption that one can detect pancreatic cancer at an early stage, at worst as pancreatic carcinoma in situ [84]. There is some evidence to suggest that those patients with pancreatic tumours of <1.0 cm can be cured. Ariyama et al. reported a 100% 5-year survival rate for seven individuals with tumours <1.0 cm and limited to the epithelium [85,86].

Certainly there is evidence that increasing tumour size correlates with an increasing rate of unresectability and decreasing survival rate underpins the need to detect tumours while they are small and have not spread locally [85]. There is also an increasingly attractive argument that the presence of high-grade dysplasia (pre-cancerous lesion) is in itself enough to justify surgery [87]. The decision to undertake surgery will be based on the risk of developing cancer outweighing the risk of an operation.

Careful characterisation of families with hereditary pancreatitis may allow trends to be established in the age of onset of pancreatic cancer. This in turn would allow the age at which pre-test risk would be enough to justify secondary screening.

Imaging of the pancreas

The most common imaging modalities at present are computed tomography (CT) and ultrasound (US) followed by endoluminal ultrasound (EUS) and positron emission tomography (PET) [88]. Alternatives are magnetic resonance imaging (MRI), endoscopic retrograde cholangiopancreatography (ERCP) and magnetic resonance cholangiopancreatography (MRCP). Little data exists on the sensitivity of these techniques in detecting lesions in asymptomatic individuals. It is clear that despite significant strides in technology, no individual imaging technique has achieved sufficient accuracy to precisely assess tumour resectability in pancreatic cancer; therefore, combinations of imaging modalities are employed. To date no consensus about the best approach to assess tumour stage or resectability has been achieved; reliable data on their combined efficacy is limited to a few prospective trials [89].

Screening modalities

Computed tomography

Traditionally, the purpose of CT has been to diagnose and stage pancreatic cancer once clinically suspected or once a patient has developed suspicious symptoms [90-92]. It has generally not been considered useful for screening asymptomatic individuals because of the belief that CT is less sensitive than EUS [93,94]. In spite of this, CT remains the most widely available and best validated tool for pancreatic imaging [95]. The sensitivity for detection of pancreatic cancer was investigated by Gangi et al. [96]. Two 'blinded' radiologists reported CT scans from patients subsequently diagnosed with pancreatic cancer. Signs of pancreatic cancer, either definitive or suspicious were identified in 93-100% of scans obtained 0-2 months before clinical diagnosis. However, with scans obtained 2-6 months and 6-18 months before diagnosis, detection was 67-83% and 63%, respectively. Only 7% of scans taken 18 months or more before diagnosis were suggestive of cancer [96]. The sensitivity of helical CT in the detection of small adenocarcinomas of the pancreas (≤2 cm) at pathological examination was evaluated by Bronstein et al. [97]. They found a sensitivity of 77% (2 observers) and 72% (10 observers) in small pancreatic masses; this group also looked at scans from patients with no adenocarcinoma and obtained a specificity of 100% (all observers). However, this high specificity is of little relevance to hereditary pancreatitis as no patients in their study had chronic pancreatitis, which may mimic carcinoma on imaging [98].

The earliest finding consistently identified by radiologists was pancreatic duct dilatation, followed by pancreatic duct cut-off [93]. These features would be expected in nearly all patients with hereditary pancreatitis, certainly with older patients (who are at most risk of cancer). Ishikawa et al. found that almost 60% of small adenocarcinomas (<1 cm) showed pancreatic duct dilatation without a mass on CT or EUS, whereas <15% showed a mass [86].

With the availability of multidetector spiral computed tomography (MDCT) scanners with narrow slice thickness and biphasic technique, the accuracy for the detection of pancreatic cancer before development has improved and despite the limitations in this group of patients it should be employed in any secondary screening programme.

Endoscopic retrograde cholangiopancreatography and molecular screening of pancreatic juice

Endoscopic retrograde cholangiopancreatography (ERCP) has a played a significant role in the diagnosis of pancreatic diseases since its development in the 1960s. According to the Japan Pancreas Society in 2003, ERCP is ranked as the third most frequent diagnostic modality employed in detecting cancers of the pancreas [99]. ERCP allows the anatomic visualisation of the hepatobiliary tree and provides a mechanism of collection of pancreatic juice for genetic analyses, brush cytology, and biopsy. Niederau and Grendell combined data from almost twenty studies and found a sensitivity of 92% and specificity of 96% for diagnosing cancer of the pancreas by ERCP [100], however, this analysis relied heavily on detection of fairly late stage tumours and the relevance to secondary screening must therefore be treated with caution.

ERCP-directed brush cytology can be used to investigate and evaluate lesions of the pancreato-hepatobiliary systems including the ampulla of Vater [101,102]. This technique requires an experienced cytopathologist and has a sensitivity, which ranges from 33-57%; the specificity ranging from 97-100% [101,103-110]. The low sensitivity may be related to technical problems and difficulties in sampling or visualisation

[111]. The role of ERCP is evolving into a therapeutic modality; its role in diagnostics is slowly being superseded by endosonographic modalities, however, the development of molecular screening models such as that developed by EUROPAC are likely to improve the sensitivity and specificity for the detection of early pancreatic cancer in high risk groups [81]. Nonetheless, the potential benefit of ERCP for pancreatic juice sampling and molecular analysis must be carefully considered against the risks involved, most significantly the risk of acute pancreatitis. Estimates of the risk of ERCP induced pancreatitis vary from 4 to 7% [112-114]. Mortality associated with post ERCP pancreatitis is approximately 0.2% [112]. These risks will depend on many factors relating to the patient and the nature of the procedure; patients with existing chronic pancreatitis would be likely to have a lower risk.

In order to improve specificity without significantly compromising sensitivity, molecular changes occurring during tumour progression are being exploited. Mutations in K-Ras occur at an early stage of development and can be found in 85% of patients with pancreatic cancer [115]. The detection of these mutations in stool and duodenal or pancreatic juice has been proposed as an early detection strategy [116]. However, K-Ras mutations can be identified in pancreatic juice from patients with chronic pancreatitis or even biliary tract stones as well as patients with cancer [81], making the specificity of this test very low. The $p16^{INK4a}$ tumour suppressor gene is inactivated in around 95% of pancreatic cancers, but this occurs later in cancer progression than K-Ras mutation [117,118]. Although, promoter hypermethylation is only involved in approximately 16% of $p16^{INK4a}$ inactivation [118], promoter methylation of DNA extracted from pancreatic juice appears to be elevated in most patients with pancreatic cancer, reflecting a change in the non-tumour cells of the diseased pancreas [81]. Detection of $p16^{INK4a}$ promoter CpG island methylation has been examined as a screening modality for pancreatic cancer [81,119-121]. Initial reports of no promoter methylation in cancer patients probably reflected low sensitivity of the assay and subsequent analysis indicated some level of promoter methylation in all pancreatic juice samples, from cancer patients or from controls [81]. Quantification rather than detection was therefore used to distinguish cancer patients, raising the threshold for the methylation level considered as positive allowed specificity to be increased but at the expense of sensitivity; a compromise threshold of 12% promoter methylation gave nearly 90% specificity with over 60% sensitivity [81].

The p53 tumour suppressor gene is mutated in about 50% of pancreatic ductal adenocarcinomas [122]. Immunocytology detects mutant p53 indirectly as a result of the accumulation of mutant p53 protein in cells. This technique however, will miss mutations that lead to loss or truncation of p53 protein [123]. Mutations have also been detected in pancreatic juice using single stranded conformational polymorphism (SSCP). In the largest of these studies, 11/26 patients (42%) with pancreatic cancer had a detectable p53 mutation in comparison to 0/16 patients with chronic pancreatitis [124]. SSCP lacks sensitivity (detecting approximately one mutant copy per 100 wild type copies) and cannot distinguish between polymorphisms, functionally silent mutations and inactivating mutations. A yeast functional assay which acts by detecting the essential transcriptional activation function of p53 has also been applied to pancreatic juice. In this technique, human p53 expressed in Saccharomyces cerevisiae activates transcription of the ADE2 gene. Yeast colonies containing wild-type p53 are white, while colonies containing mutant p53 are red as a result of the accumulation of a metabolic intermediate [81]. Using this technique 42% of 48 cancer patients were correctly identified, with no mutant p53 being identified in 49 patients with biliary tract stones (although p53 mutations were detected in 2/49 patients with chronic pancreatitis).

Recently, Yan et al. of the EUROPAC study group published data on stratification of cancer risk using p53 and K-Ras mutation status combined with $p16^{INK4a}$ promoter methylation [81]. They concluded that for individuals in a population with a 1% incidence of cancer, risk could be stratified between negligible and over 50%; exceeding 90% when discriminating patients with malignancy from patients with no pancreatic disease. The authors admit that their analysis (a Bayesian approach using the specificity and sensitivity of the three tests as independent) was based on patients with a presumed diagnosis prior to molecular analysis, which would tend to lead to an overestimate of the power of the screening modalities. Work is ongoing to clarify the sensitivity of the modalities in asymptomatic patients.

Clearly, such molecular screening models have enormous potential as adjuncts to pre-existing screening tools in the clinical management of high risk patients and are already implemented in centres like Liverpool (EUROPAC) as part of the multidisciplinary, multimodality screening programme already in place.

Endoluminal ultrasound

Endoluminal ultrasound (EUS) is high frequency, real-time ultrasonography combined with endoscopy. EUS is associated with a very low risk of adverse effects (0-0.5%) and very high sensitivity (>90%) for the detection of early, non-metastatic, pancreatic cancer [88,125,126]. As a modality, EUS can display small pancreatic lesions undetectable by CT and MRI. Some centres in the United States recommend screening for pancreatic cancer by performing yearly EUS, followed by ERCP, EUS guided fine-needle aspiration or CT to further investigate abnormalities [127]. This is in accordance with the American Gastroenterological Association recommendations for those with familial syndromes [128]. This approach has been tested with 38 high risk patients, none of whom had symptoms of pancreatic cancer. None of these patients had hereditary pancreatitis. Pancreatic masses were identified in seven patients. All seven were operated on and one of them (a 45 year old female with a history of breast cancer) was found to have an invasive ductal adenocarcinoma, this patient is still alive five years after surgery. Rulyak et al. suggested that the use of EUS to screen members of a familial pancreatic kindred was cost-effective, however, the benefit is limited to populations with a pre-test probability of pancreatic dysplasia >16% [129]. According to Rulyak et al. screening should begin at 50 years of age, or 10 years before the earliest age of onset of pancreatic cancer

in a family member, beginning with yearly examinations in a pancreatic specialist centre [130].

Given the risk of pancreatitis with ERCP, it may be reasonable to perform an EUS prior to an ERCP in patients with a family history of cancer [131]. Therefore, at the University of Washington Medical Centre, the first phase of screening in high risk patients involves EUS, which if abnormal is followed by ERCP [132]. If both are normal then they are repeated annually or per patient's choice [132]. However, it is questionable whether EUS can detect a small lesion on a background of pancreatitis and so for hereditary pancreatitis the additional modality of CT is indicated.

Tumour markers

Many of the imaging techniques previously described have the disadvantage that they are invasive or involve morbidity as a result of exposure to radiation. Therefore, a simple serum based test has advantages if adequate specificity and sensitivity can be achieved. A number of proteins have been identified that have raised levels in patients with pancreatic cancer; the question remains whether this increase occurs early enough to give the required sensitivity and whether this increase is specific to pancreatic cancer or whether levels may be elevated in high risk patients even in the absence of tumours. In addition to a high sensitivity and specificity, tumour marker testing should be cheap and reproducible.

Carbohydrate antigen 19-9 in serum

Carbohydrate antigen 19-9 (Ca19-9) is a cell surface glycoprotein (a monosialoganglioside) expressed on the surface of pancreatic cancer cells as well as by normal human pancreatic and biliary duct cells, and gastric, colonic, endometrial and salivary epithelia. It is elevated above 100 U/ml in the serum of patients with hepatocellular carcinoma, ovarian carcinoma, bronchial, colon and gastric cancers as well as pancreatic cancer. It has been found to be a useful tumour marker in diagnosis, a prognostic indicator and provides an overall evaluation of therapeutic efficacy and recurrent disease status [111,133].

Only 50% of cancers <2 cm are associated with a rise in Ca19-9 [111]. The limitations of Ca19-9 were well demonstrated in a study by Kim et al., who found a positive predictive value of less than 1% for patients undergoing ultrasonography who were described as asymptomatic; they tested 71,000 individuals using a cut-off of 37 U/ml [134]. Another important limitation of Ca19-9 relates to patients with negative Lewis blood group antigen (Lewis a-, b-). This group of patients representing 4-15% of the population are unable to synthesize Ca19-9 and so its use in this population should clearly be avoided [133,135-137].

In an early publication by Malesci et al., a Ca19-9 greater than 40 U/ml was found in 90% (57/63) of pancreatic cancer patients and in only 10% (5/50) of patients with chronic pancreatitis [138]. In 4/5 patients with chronic pancreatitis, repeat testing when the patients were in a non-relapse state revealed normal levels of Ca19-9. This study highlights that a progressive upward trend seems to be more indicative of pancreatic cancer than fluctuating levels, which may be associated with the degree of active inflammation in patients with pancreatitis [138].

Forsmark et al. retrospectively reviewed 53 patients with Ca19-9 values >90 U/ml in whom the test had been done because of clinical suspicion of pancreatic malignancy [139]. Pancreatic cancer was found in 85% (45/53) of patients. When a cut-off value of Ca19-9 >200 U/ml was used, 97% (36/37) of patients had pancreatic cancer. Thirty patients with pancreatic cancer and no radiographic criteria of unresectability underwent attempted resection; five of these patients were judged to be potentially resectable; four underwent attempted resection. In only one patient with a Ca19-9 value >300 U/ml was resection possible. Forsmark et al. concluded that pancreatic malignancy was highly suggestive in patients with suspected pancreatic cancer and a Ca19-9 >90 U/ml, while a Ca19-9 >200 U/ml was considered virtually diagnostic. In those with a Ca19-9 >300 U/ml, resection was rarely possible.

As high levels only appear to be found in late disease, use of tumour markers like Ca19-9 as a serum screening modality for the early diagnosis of pancreatic cancer is extremely limited. It should only be utilised in combination with radiological imaging and endoscopy, and molecular screening methods such as the molecular mutational analysis of pancreatic juice employed by the EUROPAC group.

Conclusions

In the last decade great strides have been made in our understanding of the molecular biology and pathophysiology of hereditary pancreatitis. This has been transcribed to the clinical setting resulting in better clinical management of those individuals with such inherited diseases of the pancreas. Indeed, the emphasis has been to identify such high risk groups for the development of pancreatic cancer through secondary screening programmes such as that offered by the EUROPAC study group in the hope that early diagnosis will lead to a 'cure' through a surgical resection. However, the benefits to the patient of embarking on such a screening programme must be considered carefully given the definite risk of morbidity and mortality associated with a pancreatic resection.

Hence, the quest for the ideal imaging and molecular modalities for the purpose of secondary screening for the diagnosis of early pancreatic cancer remains both challenging and unresolved. Philosophically, the individual's best interest must be sought in light of the latest advances in medicine and science following discussion with a multidisciplinary team inclusive of genetic counselling.

The identification of precursor lesions within pancreatic ducts has led to the formulation of a progression model of pancreatic cancer and subsequent identification of early- and late-stage changes leading to invasive cancer [93,140-145]. Ultimately, understanding the genetic events underlying the development of pancreatic cancer may serve as a useful adjunct in the screening and treatment of patients suffering from, or at risk for, pancreatic cancer. Conceptually, identification of a point on the progression, based on the appearance of molecu-

lar markers, would allow rational evaluation of the risk that cancer development is inevitable. This can only be confirmed by long-term prospective follow-up of patients from an asymptomatic state to confirmed pancreatic cancer. Prospective and repeated multimodality mutation testing of pancreatic juice in tandem with conventional imaging modalities like CT, EUS and ERCP, will further stratify the risk of pancreatic cancer in high risk groups, and thus facilitate clinical decision making.

The growth and expansion of the EUROPAC registry over the last eight years in bettering our understanding of inherited pancreatic diseases would not be possible without the collaborative efforts of both scientists and clinicians. It is only through such collaborative efforts that we may further advance our scientific knowledge of hereditary pancreatitis thus improving the management of these individuals who have an estimated lifetime risk of 40% (to the age of 70 years) for the development of pancreatic cancer [1,15]. At present, it is only through secondary screening programmes that early lesions in such high risk groups may be identified, in the hope that a curative surgical resection may be offered.

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