

Familial Pancreatic Cancer: a review and latest advances

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Abstract

Familial Pancreatic Cancer (FPC) is the autosomal dominant inheritance of a genetic predisposition to pancreatic ductal adenocarcinoma, penetrance is assumed to be high but not complete. It was first described in 1987 and since then many families have been identified, but the candidate disease gene remains elusive and the very existence of the syndrome is sometimes questioned. FPC identifies a target group for secondary screening. As well as being potentially life saving for the subjects, screening offers researchers the opportunity to elucidate the early pathogenesis of pancreatic cancer. The scientific incentive for screening should not blind us to the challenges facing clinicians in managing high risk patients. Early surgical treatment may dramatically improve the five year survival for pancreatic cancer, but this must be balanced against the risks of false positives, where healthy individuals are subjected to the mortality and morbidity of major pancreatic surgery.

Key words: Familial Pancreatic Cancer, FPC, pancreatic cancer, secondary screening, EUROPAC, Ca19-9, CT, EUS, ERCP, K-ras, p53, p16, BRCA2, anticipation.

Introduction

Familial Pancreatic Cancer (FPC) is the term used to describe the occurrence of multiple cases of pancreatic cancer within families in a pattern consistent with autosomal dominant

inheritance. FPC was initially described in 1987 with the first cohort of FPC families presented in 1989 [1]. The emerging evidence for FPC and the potential implications for research into the pathogenesis of pancreatic cancer prompted the establishment of FPC registries around the world. The authors are closely associated with the European Registry of Hereditary Pancreatitis and Familial Pancreatic Cancer (EUROPAC), which was established in 1997.

The definition of FPC has gradually been strengthened by the registries to exclude families that belong to other cancer syndromes, which carry a predisposition to pancreatic cancer (e.g. breast-ovarian syndrome), or hereditary illnesses such as hereditary pancreatitis (HP), which carry an increased pancreatic cancer risk [2]. The exact proportion of pancreatic cancer deaths that are linked to inherited genetic factors remains uncertain although it has been estimated as 10% [3,4].

Early symptoms are subtle and non-specific and by the time the disease presents clinically, the vast majority of pancreatic cancers can only be treated with palliative intent. For patients diagnosed in the UK between 1996 and 1999, five year survival was between 1.7 and 3.5% [5]. There is evidence that this survival rate is improving, based on better treatment modalities for the minority of patients that have resectable disease [6]; Jemal *et al.* estimated the number of new cases of pancreatic cancer in the US in 2007 to be 37,000 [7] with only 33,000 deaths.

Segregation analysis of FPC families suggests a rare major gene conferring predisposition [8], whilst other studies claim an autosomal dominant transmission [9]. Autosomal dominant transmission remains controversial, but mechanistically this is the most likely form of transmission given a single major gene. Inherited predisposition for cancer is usually the result of a heterozygous defect in a tumour suppressor, loss of the second copy of the tumour suppressor being the second "hit". Genetic instability is part of the ageing process and so the second hit will be inevitable if an individual lives long enough.

In the post-genomic world, a genetic syndrome will only truly be accepted once a mutation segregating with the disease

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is identified. In 20% of FPC families a mutation in the BRCA2 gene has been shown to segregate with the disease [49], but for the majority of FPC families, no disease gene has been identified. It is quite possible to have more than one case of pancreatic cancer in a family without any particular genetic predisposition. Thus selection of families retrospectively, on the basis of multiple cases of cancer, could give a false appearance of an autosomal dominant disease.

Potential causes of artefactual familial clustering of pancreatic cancer

To qualify as an FPC kindred on the EUROPAC registry, families need to have at least two proven cases of pancreatic cancer that are consistent with high penetrant autosomal dominant inheritance. For example, a family with pancreatic cancer in both a father and son could be classified as FPC if at least one paternal grandparent died at a reasonably young age (EUROPAC classify this as below the age of 75), but if both paternal grandparents were over the age of 75, then the family would not be classed as FPC. The unavoidable consequence of this is that a kindred with two cancers can be classified as FPC on the basis of an incomplete pedigree, only to be reclassified, as grandparents and great grandparents are added to the family tree. The overall lifetime risk of developing pancreatic cancer for the general population is 0.5-1% [10]. By definition, the chance of developing pancreatic cancer in an FPC kindred approaches 50%. Even so, it is not inconceivable that a large family could have two cases by chance alone.

Selection Bias

Pancreatic cancer patients in the USA were asked to report any other cases in first degree relatives. Approximately one in ten were able to do so [11,12]. The chance of identifying two cases will be determined by the number and ages of individuals in the kindred, furthermore, in these studies all the families will include at least one pancreatic cancer case (the proband), so the chance of two cases in one of these families is roughly equivalent to the chance of finding a single case in an unselected kindred. Case-control studies are desirable, although of necessity more difficult to carry out. In a study of individuals under the age of 75 admitted to Ospedale Maggiore [13], the relative risk of a pancreatic cancer patient reporting a first degree relative with pancreatic cancer was 3.3 fold that of controls (95% CI 1.42-2.44). This was based on 14 cancers in 362 families with the proband suffering from cancer compared to 15 cancers in 1408 control families. A subsequent American study indicated very similar relative risks (3.2, 95% CI: 1.8-5.6) [3] comparing the families of 484 pancreatic cancer cases with the families of 2099 controls. Familial studies like these are open to criticism as they are affected by the size and closeness of relationships within the family kindreds and there is no easy way to control for this.

Genetic Factors

The relative influence of genes and environment is a notoriously difficult area, people who share common genetic backgrounds often have similar diets, occupations and customs. Pancreatic cancer has been shown to be more common in black than white Americans [14]; this could be due to low penetrance or multigene susceptibility, or simply that black Americans lead a lifestyle that is more "high risk". In support of an environmental rather than genetic link, migration studies show that pancreatic cancer risk amongst Japanese migrants moving to the US increases and overtakes the level of cancer risk of white Americans [15]. The most likely cause of this is the Japanese adopting the "Western" high meat, high fat diet. However, a direct link between Western diet and pancreatic cancer has not been shown despite large cohort studies [16]. An indirect link via obesity and diabetes (see below) cannot be ruled out, but neither is there any evidence that it explains the migration studies.

Gender

Analysis of the Surveillance Epidemiology and End Results (SEER) data [10] shows a slightly greater incidence of pancreatic cancer in men than women (see *Fig. 1a*). The SEER data is cross-sectional, while familial data, such as that held by familial pancreatic cancer registries is, by definition, longitudinal. To compare the two sets of data it is either necessary to model longitudinal data using the SEER figures or to take a date for a cross-sectional study of the registry data. A comparison has been carried out in *Fig. 1a* with data from the EUROPAC registry, taking individuals alive in the year 2000 and using a five year window for occurrence of pancreatic cancer. The SEER data shows a clear higher incidence of pancreatic cancer for men in all age groups, the data is far less clear cut for the EUROPAC data, although this could be because of the small numbers of at risk individuals in each age group. Overall, in the EUROPAC families death from pancreatic cancer does occur slightly earlier in males, this is shown in the Kaplan-Meier curve in *Fig. 1b*. However, the final lifetime risk for men and women is roughly equivalent in this population (approximately 50%). This is in stark contrast to the situation in sporadic disease.

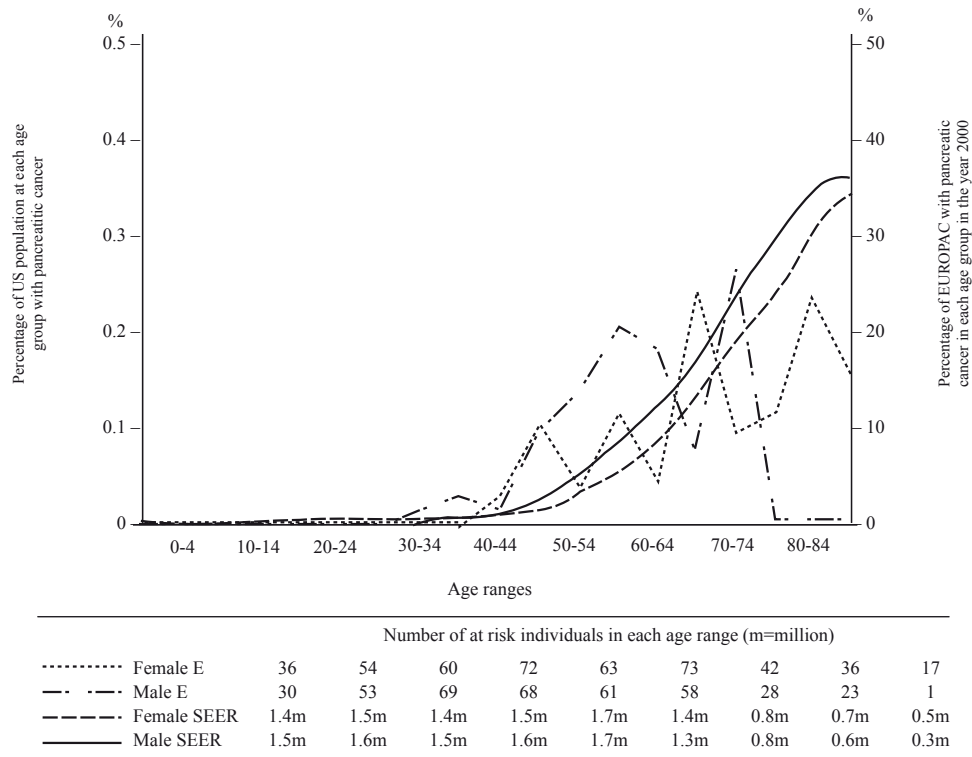
Environmental and Lifestyle Factors

The best evidence for a link between an environmental risk factor and incidence of pancreatic cancer exists for tobacco smoking [17]. Overall, smoking increases the risk of pancreatic cancer by two-fold [18], with some evidence for a dose-response relationship [19]. The risk posed by passive smoking remains unproven, thus clustering of pancreatic cancer within families is more likely to be related to a common habit shared by family members, than contamination of the family home by a single heavy smoker. Analysis of the EUROPAC database has shown no direct evidence for smoking as the cause of familial clusters of pancreatic cancer [9].

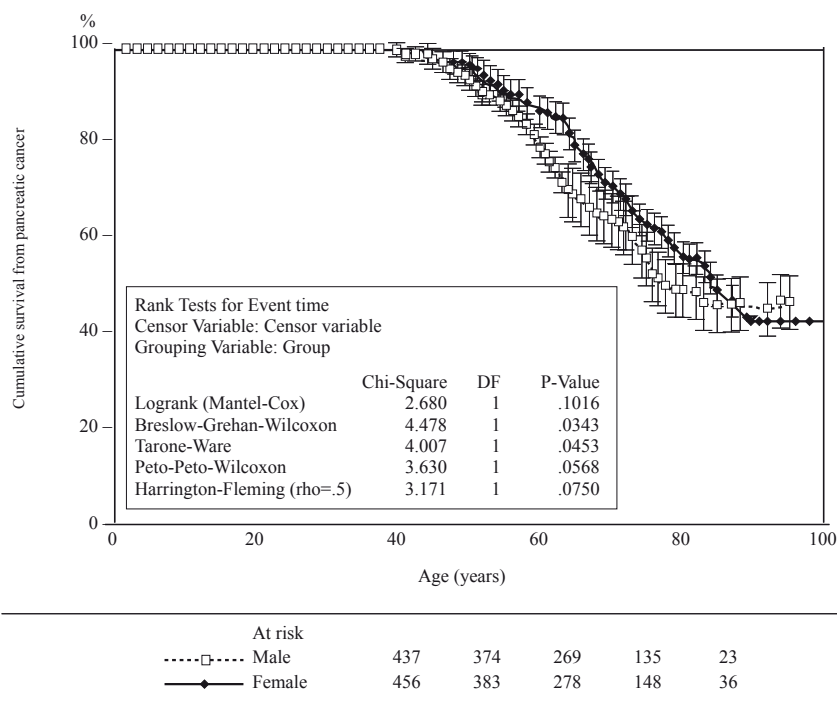
Figure 1. Gender and risk of pancreatic cancer in FPC families and sporadic disease.

In 1a the incidence of pancreatic cancer for each gender is plotted for 5 year age groups using the SEER data, more men develop pancreatic cancer than women in each age group. This is compared to data from the EUROPAC database using a separate scale, taking the age of individuals alive in 2000 and following for pancreatic cancer until 2005. The number of individuals taken for the analysis are given below the graph (E=EUROPAC). There is a trend for a higher percentage of men to develop pancreatic cancer in the earlier age groups, but the small number in each group makes comparison difficult. In 1b a survival curve is plotted for the EUROPAC data, women develop pancreatic cancer significantly later, but overall lifetime risk is equivalent (data as used in McFaul et al. [9])

1a



1b



A link has been shown between pancreatic cancer and obesity [20]. Obesity shows familial clustering, thought to be due to shared behaviours, so this may contribute to some cases classified as FPC.

There has been particular emphasis on searching for a link between pancreatic cancer and occupations that lead to contact with chlorinated hydrocarbons, especially dichlorodiphenyl-trichloroethane (DDT), though no definite link has been established [21,22].

Associated Medical Conditions

There are two major illnesses linked to pancreatic cancer; diabetes mellitus and chronic pancreatitis. Some 80% of pancreatic cancer patients have impaired glucose metabolism. Tumours can induce production of diabetogenic peptides which result in insulin resistance reminiscent of type 2 diabetes [23], this can often be alleviated by resection of the tumour [24]. In sporadic disease development of higher baseline fasting glucose levels appears to be a very early symptom of pancreatic cancer [25] but this has not been shown in familial pancreatic cancer patients. It is also possible that diabetes is a risk factor, as well as a symptom, of pancreatic cancer but this remains unproven [24]. Diabetes shows familial clustering and is a feature of Family X, one of the best characterised of all FPC families [26]. It is possible that diabetes could explain some cases classified as FPC on the EUROPAC database, but an analysis has failed to show an increased incidence of diabetes mellitus, above that expected as a symptom of pancreatic cancer.

A second possible cause of familial clusters could be multiple cases of chronic pancreatitis within a family. This could be caused by a shared tendency to heavy alcohol intake or the rare genetic syndrome, hereditary pancreatitis. Chronic pancreatitis has been shown to lead to a 15% lifetime risk of pancreatic cancer [27] and the cumulative lifetime risk increases to 35-40% in hereditary pancreatitis families [28,29].

Interaction of Genetic and Environmental Factors

It is conceivable that multiple cases of pancreatic cancer in a family could be caused by genetic variations other than the elusive FPC mutation that could possibly increase the impact of environmental factors. Such variations, although inherited, would not justify the description of FPC, as the link is indirect and the elevation in risk should not give the prospective appearance of autosomal dominant inheritance of pancreatic cancer. Genetic polymorphisms have already been linked to the development of pancreatic cancer. Pancreatic adenocarcinoma was shown to be associated with the UGT1A7*3 allele of UDP-glucuronosyltransferase, an enzyme known to be involved in detoxifying tobacco carcinogens [30]. Both thymidylate synthase and methylenetetrahydrofolate reductase promoters have a direct association with occurrence of pancreatic cancer [31], a surprising observation as interest in these genes was based on the assumption that they would influence response to chemo-

therapeutics rather than incidence. Developments in SNP-based array technology and a more empirical approach will allow further predisposing polymorphisms to be identified. However, as over 90% of the population has a very small risk of pancreatic cancer, it is unlikely that any commonly occurring polymorphism would cause a sufficient increase in risk to account for FPC and a rare combination of multiple unlinked polymorphisms should not lead to a family history of pancreatic cancer covering more than one generation.

Cystic Fibrosis (CF) affects multiple systems by causing obstruction of ducts; one organ affected is the pancreas. Two early onset cases of pancreatic cancer were identified in 28,000 cases of cystic fibrosis (odds ratio 31.5 vs control group) [32]. CF is a recessive disease but there are a number of clinical implications for heterozygotes with mutations in the cystic fibrosis transmembrane receptor (CFTR) gene; amongst these is a greatly increased risk of chronic pancreatitis [33,34] and so it is at least conceivable that a similar autosomal dominant inheritance of pancreatic cancer risk may be observed under certain circumstances, or with specific CFTR mutations. A study of 166 early onset pancreatic cancer patients (under the age of 60) found 14 carriers of disease related CFTR mutations (8.4%) compared to 4.1% in controls (odds ratio 2.18, 95% CI: 1.24-3.29) [35]. None of the 14 cancer patients had a family history of pancreatic cancer, which is unsurprising given the fairly modest increased risk.

Autosomal dominant inheritance of a predisposition to other forms of cancer is well known, for example colorectal cancer in hereditary non polyposis colorectal cancer or breast cancer in breast ovarian syndrome. In many cases the disease mutations have been identified by linkage and sequencing of candidate genes, furthermore these mutations have been shown to be common to many families. These inherited syndromes often have a spectrum of cancer sites; some of them include an elevated risk of pancreatic cancer. Therefore, it is conceivable that by chance a family with a more general syndrome will present with pancreatic cancer cases in the absence of other tumours. It should also be understood that although FPC is defined specifically in terms of pancreatic ductal adenocarcinoma, it is possible that ampullary tumours, extrahepatic cholangiocarcinomas, acinar cell tumours and even pancreatic neuroendocrine tumours may have been included due to misdiagnosis when defining a family, accounting for part or all of a familial cluster. It is even possible that misdiagnosis, or misreporting of colorectal or gastric tumours may explain part of a cluster.

Registries such as EUROPAC, the National Familial Pancreas Tumor Registry (NFPTR) and the German National Case Collection for Familial Pancreatic Cancer (FaPaCa) require reliable evidence of pancreatic ductal adenocarcinoma before registering a family. This means that highly penetrant syndromes with known disease mutations are unlikely to be confused with FPC. For example mutations in the VHL gene which cause von Hippel-Lindau syndrome are associated with pancreatic neuroendocrine tumours [36], only occasional pancreatic ductal adenocarcinoma have been reported in these families [37]. Li-Fraumeni Syndrome is associated with p53 and CHK2 mutations. At least 24 families have been reported with multiple cases of pancreatic cancer, which superficially would

be consistent with FPC [38]. However, in the same study the families were followed for 10 years and over 200 cases of non-pancreatic cancer were reported [38]. It is unlikely that such an extreme cancer risk would be missed by even the most cursory family analysis and so such families would not be included as FPC by any of the large registries. Another example, is Peutz-Jeghers syndrome (PJS); autosomal dominant inheritance of hamartomatous polyposis. The reported increased risk for pancreatic cancer is very great (132 fold) [39], this is an adequate level to give multiple cases within a family. However, in the largest study of PJS only 6 pancreatic cancer patients were reported. The reason for the small number of cases is the high mortality from other cancers in these families, so as for Li-Fraumeni it is very unlikely that a PJS family will be mistaken for FPC [40,41]. EUROPAC originally had a policy of screening possible FPC families for the STK11 mutations that cause PJS, but no mutations were identified [42].

Similarly, low penetrance cancer syndromes associated with well defined phenotypes other than cancer would be unlikely to be confused with FPC. For example, mutations in the ATM gene cause ataxia-telangiectasia, an autosomal recessive inherited disease characterised by oculocutaneous telangiectasias, cerebellar ataxia and cellular and humoral immune deficiencies. People with ataxia-telangiectasia have increased cancer risk, estimated at 50 to 150-fold, but this would clearly be a recessive risk. Heterozygotes for ATM mutations have an approximately 3-fold increase in risk [43]. The specific risk for pancreatic cancer is at most marginal [44], it is unlikely that such a low increased risk would give many familial clusters of pancreatic cancer and even if this did occur, a familial history of ataxia would be likely. Familial adenomatous polyposis (FAP), which is caused by a mutation of the tumour suppressor gene APC, is characterised by the presence of multiple adenomatous polyps within the gastrointestinal tract. The colon is the most commonly affected site and there is a high incidence of colon cancer. The elevation in risk of pancreatic cancer is relatively small, 4.46 (95% CI: 1.2-11.4) or 21.4 cases per 100 000 person years [45]. Although it is possible that a family would contain multiple cases of pancreatic cancer, due to the numbers of colonic cases, an FAP family would be unlikely to be diagnosed as an FPC kindred.

Although the majority of cancer syndromes are unlikely to be confused with FPC by major registries, there appears to be heterogeneity in the phenotype associated with certain mutations, to such an extent that the same mutation may give a well defined syndrome in one family but give a very different phenotype in another. For example, hereditary non-polyposis colorectal cancer (HNPCC) can be divided into two groups (Lynch syndromes I and II). Both syndromes result from mutations in mismatch repair genes but Lynch syndrome I is almost exclusively associated with colorectal cancer whilst Lynch syndrome II features extra-colonic tumours in sites such as the stomach, breasts, uterus, bladder and small bowel; this group shows a clearly elevated risk for pancreatic cancer [46]. Another example is mutation of the BRCA2 gene. This can lead to an autosomal recessive syndrome associated with lymphomas and hepatomas (Fanconi Anaemia), in most cases these families have no noticeable increased risk for pancreatic or breast

cancer [47]. In other families BRCA2 mutations are associated with autosomal dominant predisposition for breast and ovarian cancer [48]. Furthermore, other families have an autosomal dominant predisposition for pancreatic cancer without any elevated risk of breast cancer. The latter example includes families that have been defined as FPC [49]. Mutation of the CDKN2A (INK4a^{p16}) gene is associated with multiple naevi and cases of melanoma, a syndrome known as Familial Atypical Multiple Mole Melanoma (FAMMM) [50]. In other CDKN2A families there are also one or more cases of pancreatic cancer, this has been described as a separate syndrome (FAMMM-PC, OMIM #606719). To date all FAMMM-PC families have included cases of melanoma, hence the probability of confusion with FPC is low. Testing of genuine FPC families has yet to identify any CDKN2A mutations [51].

The evidence for FPC

Epidemiological evidence

The sheer number of families that are included in registries provides strong evidence pointing towards FPC as a genuine genetically defined syndrome. EUROPAC has registered 250 families with multiple cases of pancreatic cancer, of which 83 are consistent with a specific autosomal dominant predisposition for pancreatic cancer; the remaining families can be explained by the causes of clustering outlined above. Within these 83 families there are no obvious non-genetic risk factors. Although inclusion of some artefactual families cannot be ruled out, the rigorous evidence required to meet the strict inclusion criteria would tend to result in omission of many genuine families, so the incidence of the syndrome may be underestimated. It is likely that the nature of pancreatic cancer in FPC is different from that seen in sporadic cases, but to date no obvious earlier or later onset has been described and differences in molecular biology are still under investigation.

Although, on average, age of onset is similar to that seen in sporadic disease, one phenomenon that has been discovered is "anticipation" [12,52]. In simple terms, the age of onset of pancreatic cancer within FPC families occurs at an increasingly young age in consecutive generations. The fact that average age of onset remains consistent with the sporadic disease is explained by earlier generations having a later age of onset than is normal, compensated for by the younger onset in later generations. This could be explained by various forms of bias, but meticulous statistical analysis suggests that the phenomenon is real [9].

The Genetic Evidence

Identification of the gene responsible for FPC requires a mutation that segregates with the disease. For most genetic syndromes linkage analysis has been used to identify such mutations, but FPC presents particular problems when applying such an approach. Pancreatic cancer is a late onset disease making it difficult to distinguish a carrier who is yet to develop cancer, from a family member who is not carrying the mutation. Ethical and logistical reasons make it impractical to obtain samples from every family member prior to an individual developing the

disease. Once a family member is diagnosed, there is only a very short window of opportunity for research groups to approach patients for DNA, at a time of great stress or denial for those affected. This makes conventional linkage studies very difficult and as a consequence most work has concentrated on candidate genes. Various candidates have been suggested but these have either been found not to be mutated in FPC kindreds, such as STK11 [42], RNASEL [53] and various Fanconi anaemia genes [54], or they are only associated with pancreatic cancer as part of more general cancer syndromes, such as CDKN2A [51] and mismatch repair genes [55]. The only exception has been a small number of families which are entirely consistent with FPC which carry BRCA2 mutations [49]. The lack of progress that has been made using candidate genes has prompted a return, despite its problems, to conventional linkage analysis.

To overcome the problem of identifying carriers, Brentnall *et al.* used a surrogate of pancreatic dysplasia for pancreatic cancer. Patients with dysplasia were identified by screening within Family X, a large family characterised by a high incidence of diabetes as well as pancreatic cancer. Using this approach they were able to identify a region at the end of chromosome 4 which gave two point LOD scores of greater than 3, with three point LOD scores reaching a maximum value of 5.36 [26]. The minimum defined area was 4q32-34 and the same group have now provided evidence that the disease mutation for this family lies within the palladin gene [56]. However, recent work from the EUROPAC/FaPaCa study groups [57] and the NFPTC [58] suggest that the 4q32-34 locus is unlikely to account for a significant proportion of families and the palladin mutation has not been identified in other FPC kindreds [59,60].

Work is ongoing in a number of institutions, exploiting novel mathematical models to account for the ambiguity in defining carrier status [9] and new technology, such as SNP arrays, to increase the efficiency of linkage and association studies [61].

Management of risk in FPC

What is the risk?

The definition of FPC as an autosomal dominant condition suggests that risk is equivalent to penetrance, however, this is complicated by the issues of misclassification (as discussed above) and the lack of a recognised disease mutation in most families. It is assumed that penetrance in FPC is high, but not 100%. If penetrance in FPC were 80% to 75 years, then lifetime risk for a mutation carrier would be 80%. The risk to an individual in the same family without a mutation would be that of the general population (0.5-1%) [62]. In the absence of a test for mutation status in most families, the lifetime risk can only be estimated on the basis of some form of probability calculation giving the perceived chance that the individual is a mutation carrier. For example, half of all first degree relatives of pancreatic cancer patients in a genuine FPC family would be mutation carriers; on the basis of 80% penetrance they would therefore be estimated to have a 40% lifetime risk. On the discovery of a disease mutation the estimation of risk for these same individuals would rise to 80% or fall to that of the general population.

This does not take into account the possibility that the family only appears to be FPC. An attempt at risk quantification was performed by Klein *et al.* [63]. A prospective registry-based analysis showed that members of families with one confirmed pancreatic cancer death had a 4.6 fold increase in risk over the general population. If there were two confirmed cases the risk increased to 6.4 fold and was increased 32-fold in families with three affected members. Ignoring low penetrance conditions, this equates to estimation of the likelihood that an individual is a member of an FPC family.

Lifetime risk is a poor measure when considering the possibility of screening to identify cancer cases. As will be discussed below, the benefits of identifying an early cancer must be balanced against the loss of quality of life adjusted years as a result of the morbidity and mortality associated with screening and surgery. In order to make a rational decision on the benefits of screening, the short term risk of cancer is much more relevant. Data from the EUROPAC study group was graphed against the SEER data from the United States of America (*Fig. 2a*). This suggests a constant increased risk for all age groups, approximately equating to a 120 fold increase in normal risk (*Fig. 2b*). Even with a 120 fold increase, risk below the age of 40 is negligible. On this basis, EUROPAC only propose screening after the age of 40, although exceptions are made on the basis of anticipation.

Screening Tools

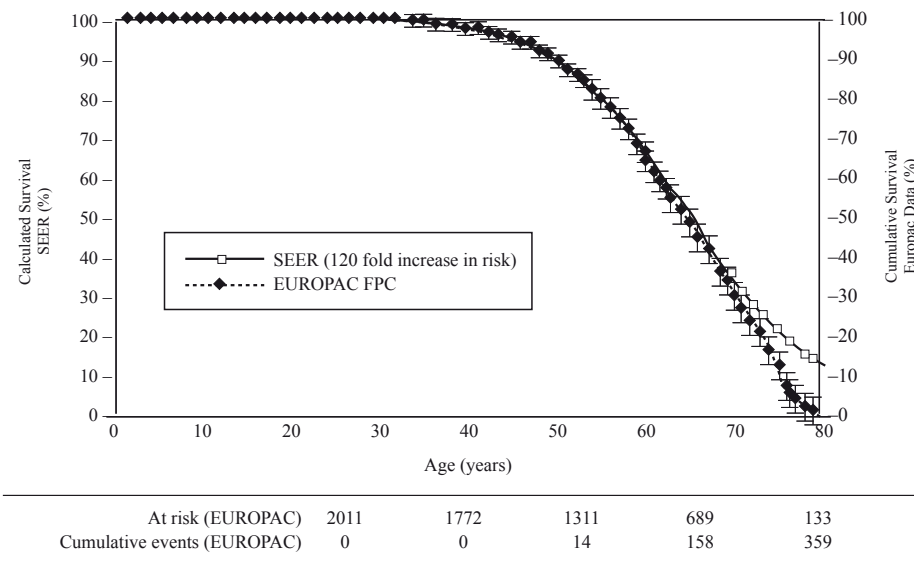
The identification of the high risk group is another way of saying "primary screening". This is predominantly achieved by careful history taking and confirming causes of death using histological records or cancer registry information. The attempt at diagnosing emerging pancreatic cancers within these groups is "secondary screening". Members of FPC kindreds are increasingly well informed and generally realise they have an elevated cancer risk, not surprisingly this causes anxiety and a demand for some form of surveillance.

Screening for pancreatic cancer is particularly challenging because the blood testing and imaging available are insufficiently sensitive and specific to detect curable pancreatic tumours without an unacceptably high number of false positives. Unlike many other high risk groups, members of FPC families are generally healthy when they approach clinicians for screening. A positive test could result in major pancreatic surgery, which carries a perioperative mortality rate of approximately 4%, even at the best centres [64]. In addition to the risk of death, any false positives could lead to resection of healthy pancreatic tissue rendering the patient dependent on pancreatic enzyme supplementation and insulin for life.

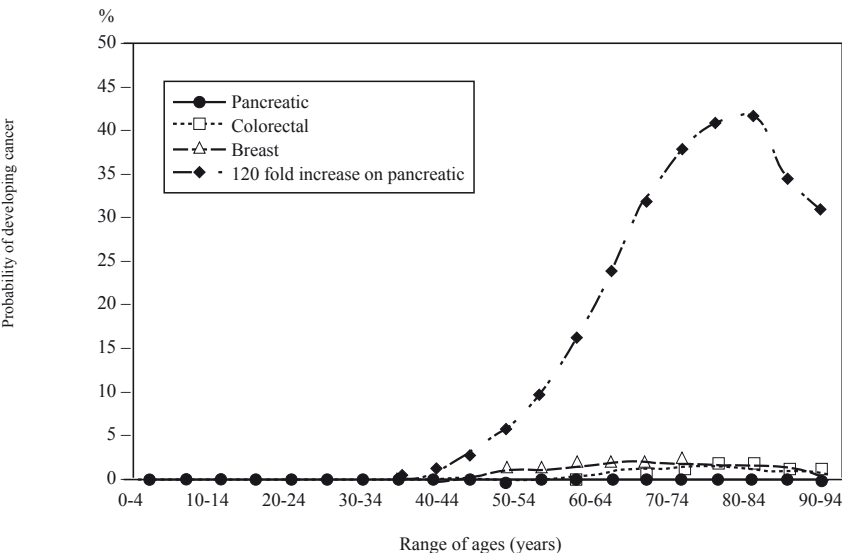
In addition to the morbidity of the operation there is also morbidity associated with the screening modalities. Ideally the screen should be safe and non-invasive, in practice the closest that is possible to this ideal, is a serum test. Many such tests have been proposed, the most commonly applied diagnostically is serum Ca 19-9. This is a sialylated Lewis antigen produced by patients with digestive tract cancers, particularly those of the pancreas and biliary tree. Estimates of sensitivity and specificity vary depending on the size and stage of the tumours

Figure 2. Age specific risk of pancreatic cancer in families with FPC. The Surveillance Epidemiology and End Results (SEER) survey in the USA has provided cross-sectional data on cancer risk in five year ranges. This can be used to model longitudinal cumulative survival, allowing comparison with a Kaplan-Meier survival curve. Approximately 50% of the FPC family members are predicted to be mutation carriers, meaning minimum survival from pancreatic cancer of all potential carriers should be approximately 50%. The model of the SEER data matches with survival from pancreatic cancer produced with EUROPAC data (A) if each 5 year risk is increased by 120 fold (B) (data as used in Greenhalf et al. [103])

2a



2b



and the nature of control groups. Estimates of sensitivity in the literature range from 67-92% and specificity ranges from 68-92% [65-68]. These values were all obtained using samples from symptomatic patients, when used as a screening modality these figures would be far worse. Only 50% of cancers <2 cm are associated with a rise in Ca19-9 [69] and it is rarely elevated in the presence of dysplasia [70]. The obvious limitations mean that it cannot be used in isolation in a screening context. In a study of 71,000 patients described as asymptomatic undergoing transabdominal ultrasonography, CA19-9 was found to

have a positive predictive value of less than 1% [71]. Other serum blood tests such as Carcinoembryonic antigen (CEA), DU-PAN-2, CA 50, SLX (sialyl difucosyl Lex), ST-439 (sialyl Lex-Tn) and CA 125 are also not applicable as single modality screening tests [72]. The EUROPAC study group take a serum fasting glucose from patients on entry to the screening programme and as part of the screening cycle. Fasting glucose is a known marker for early cancer in sporadic cases, although this has yet to be proven in familial pancreatic cancer [25]. New serum markers are under investigation.

There are a number of imaging tests available. Each has its unique advantages and disadvantages. The simplest method is transabdominal ultrasound (US). It is non-invasive, readily acceptable and involves no ionising radiation. However, the physical distance from the abdominal wall to the pancreas and the number of tissue interfaces involved requires the use of low frequencies, limiting the picture quality. Whilst the sensitivity of transabdominal ultrasound in the detection of pancreatic cancer is 95% in tumours >3 cm, it reduces dramatically with smaller tumours [73,74]. Nevertheless, the advantages of US mean that it has been applied for screening. Periodic US checks were performed by Tanaka *et al.* in a group of high-risk patients. Patients over 35 years old were recruited on the basis of pancreatic duct dilatation, pancreatic cysts and common bile duct dilatation [75]. Serum amylase, elastase-I, alkaline phosphatase, bilirubin, fasting glucose, Ca19-9, CEA and a pancreas-specific US were carried out every three or six months. Any abnormality prompted a CT or ERCP with pancreatic juice collection. Of the 393 patients enrolled, pancreatic cancer was diagnosed in 41 patients. Eighteen patients had a surgical resection, three of which turned out to be false positives. Despite these encouraging figures, screening was not necessarily of benefit to these patients. Only four patients had stage I disease at diagnosis and one of these died within three years despite treatment [75].

Computed Tomography (CT) produces a three dimensional image of the pancreas using a computer to convert information derived by conventional Roentgen principles. There is evidence to support its use in the detection of early pancreatic tumours. One paper described its diagnostic accuracy for this to be as high as 85-90% [76], although other papers have found CT less useful giving a sensitivity of 69-83% and a specificity of 59-93% [77-79]. There is a significant reduction in specificity in the presence of chronic pancreatitis and CT has insufficient resolution to detect PanIN lesions. CT scanning also carries the disadvantage of exposing the patient to 10 mSv of radiation for each abdominal CT performed [80]. With at least some FPC kindreds shown to have a DNA repair defect (BRCA2) [49], the repeated use of ionising radiation to image the pancreas needs to be thought through carefully.

Magnetic Resonance Imaging (MRI) has many of the advantages of CT scanning. It is fast, non-invasive and produces a three dimensional image of the anatomy of the pancreas. MRI has the advantage that it does not involve the use of ionising radiation, but the low resolution and the large number of artefacts produced with movement have in the past limited its use [81]. Recent advances in MRI have improved the imaging of pancreas cancer, the contrast agent mangafodipir trisodium enhances normal pancreatic parenchyma but not neoplasms [82,83]. It has even been reported that T1 weighted spin-echo MRI can be superior to spiral CT imaging for detection of small lesions [83]. The reported sensitivity of MRI ranges from 83-87% and specificity 81-100% [79,84,85].

Endoluminal Ultrasound (EUS) is the imaging method of choice in patients with healthy pancreatic tissue. EUS is low risk and has a very high sensitivity (> 90%) for the detection of pancreatic masses, even in patients with very early tumours [83,86,87]. It has been suggested that the changes consistent with precancerous lesions can also be detected, making it ideal

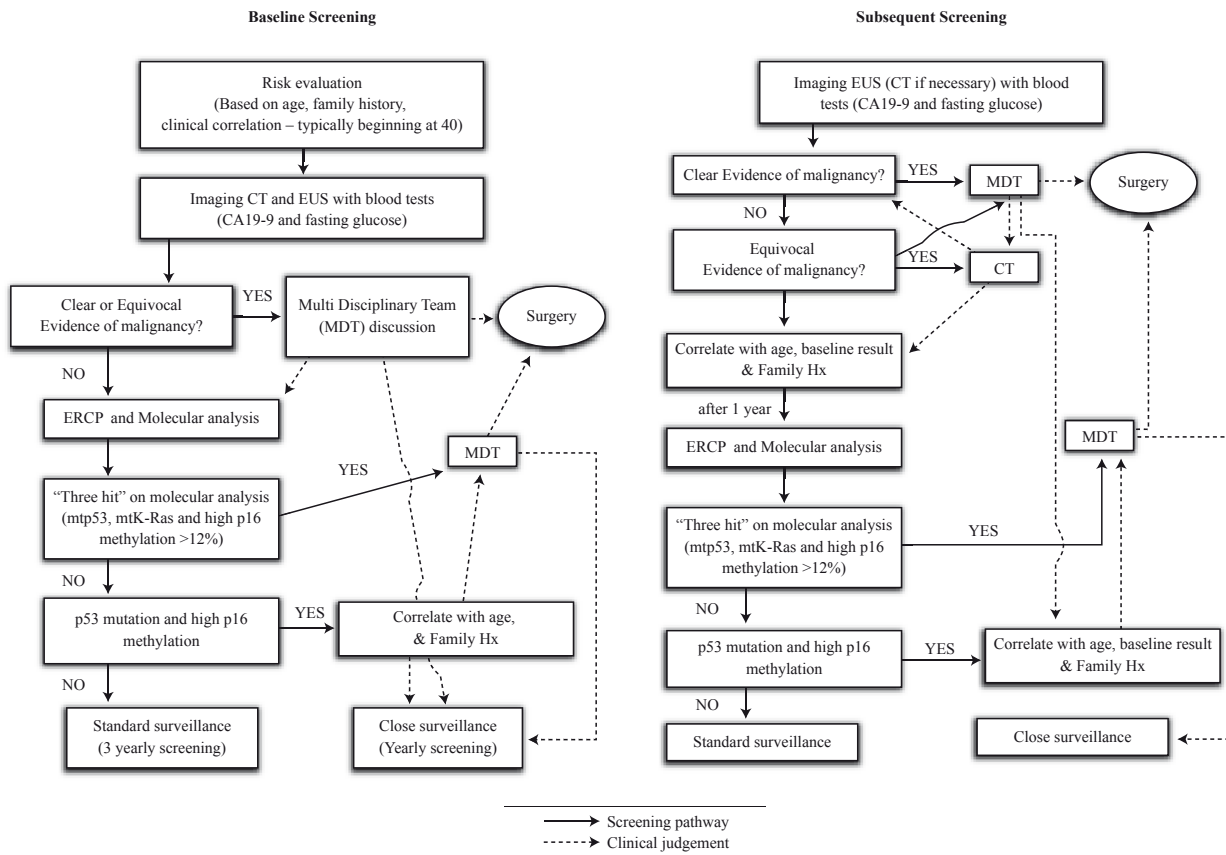
for a screening programme in high risk groups. Changes in duct histology and cytology have been observed in patients with tumours and it is widely assumed that there is a progression from normal duct architecture, through various morphological stages leading to carcinoma. These morphological changes are called pancreatic intraepithelial neoplasms (PanINs) which are numbered from 1a to 3 according to increasing abnormality. PanIN 1a lesions are little more than elongation of the ductal cells whilst PanIN 3 lesions have large displaced nuclei and are papillary, often budding off into the lumen of the duct. PanIN lesions may cause parenchymal heterogeneity, which can be visualised using EUS as echogenic foci and hypoechoic nodules. There is also a suggestion that mucin changes resulting from PanINs cause the main pancreatic duct to become hyperechoic [88]. Other lesions commonly assumed to be associated with development of pancreatic tumours are Intraepithelial Mucinous Neoplasms. These can be visualised as cystic masses [89].

EUS is not good at distinguishing between benign lesions and cancers. In a small study (n=85) aimed at distinguishing between chronic pancreatitis and pancreatic cancer, positive predictive value was only 60% based on imaging alone [90]. To improve specificity, EUS has been used to guide fine needle aspiration (FNA) or "Tru-cut" biopsy from pancreatic lesions. A study in Birmingham, Alabama conducted 300 consecutive EUS-FNA procedures on patients referred with a suspicion of cancer. It showed that diagnosis of cancer in the presence of background pancreatitis remains problematic. Of 22 patients with cancer and chronic pancreatitis, EUS-FNA detected only 14 (64%). In the absence of pancreatitis 180 out of 188 (96%) cancers were successfully diagnosed [91]. These data were obtained with symptomatic patients. It is reasonable to assume that figures would have been worse with asymptomatic cancers.

Endoscopic retrograde cholangiopancreatography (ERCP) has traditionally been used for advanced pancreatic disease and has a crucial role in the management of obstructive jaundice, with the potential to obtain cytology or place stents. A tumour causing a marked stricture could normally be visualised by less invasive modalities. For screening, interest has focused on identification of irregular or ectatic ducts with possible sacculations which are said to be associated with PanINs [88]. These changes normally occur in the side branches or in the tail of the pancreas and require an expert radiologist to perform and interpret. An alternative approach is molecular analysis of pancreatic juice obtained at ERCP [92]. Pancreatic juice is the secretion most intimately in contact with tumours and so may contain either tumour cells sloughed from the duct or cell components, including DNA, from necrotic cancer cells. This approach is only suitable for selected patients on a research basis as the potential benefits must be weighed against the risk of inducing acute pancreatitis during ERCP [93].

Magnetic Resonance Cholangiopancreatography (MRCP) is a non-invasive method of imaging the biliary tree and avoids the risks associated with ERCP. In a prospective study of MRCP using 124 patients referred with a suspicion of malignancy (37 of whom went on to develop pancreatic cancer), Adamek *et al.* found a sensitivity of 84% and a specificity of 94% [94]. Some studies have stressed the value of secretin administration in improving pancreatic ductal details in MRCP [95], but whilst

Figure 3. The EUROPAC screening protocol



MRCP is a useful, non-invasive tool in the diagnosis of pan-creato-biliary obstruction, it has not been fully evaluated in the context of secondary screening. The limited sensitivity even with symptomatic tumours suggests it has limited use as a modality in this context.

There is no single serum or imaging test that is sensitive and specific enough to be used in isolation for screening. By combining investigations it may be possible to improve sensitivity and specificity of the overall process, but there are also cost and safety issues relating to screening that need to be addressed. One possibility is to further stratify risk, increasing the pre-test incidence and avoiding unnecessary screening of relatively low risk participants. Stratification according to smoking status, diabetes or gender may all contribute to this, but is unlikely to make an adequate difference. More effective stratification can be obtained by monitoring the presence of cancer related nucleic acid or protein changes in pancreatic juice. Modalities for molecular analysis of pancreatic juice have evolved since the early experiments showing that K-Ras mutations can be detected in cellular material obtained at ERCP [96]. K-Ras is almost ubiquitous in pancreatic cancer [98], but unfortunately it was soon established that K-Ras mutations are also common in the pancreatic juice of control patients [99]. Technical difficulties have restricted detection of p53 mutations as a modality for screening, despite a high proportion of p53 mutations in pancreatic tumours and an apparent high specificity for cancer [99]. Various other markers have been investigated including telomerase expression and methylation of specific promoter

sequences. Most of these have shown promise, but this has not been sufficient to justify their inclusion as an independent screening modality [100]. EUROPAC has proposed a combination of different molecular tests to phase their screening programme. The technical aspects of the methods were published in 2005 [92], cell free pancreatic juice samples are analysed for presence of K-ras and p53 mutations and quantification of p16 promoter methylation. It was proposed that a combination of results with the three molecular tests could stratify risk between negligible and 90% probability of cancer. Stratification is less marked in patient groups with a background of pancreatitis (approximately 0 to 50%), but molecular analysis may conversely have the most impact in HP patients where the sensitivity and specificity of conventional imaging is limited [90]. The techniques have yet to be proven in a prospective study and currently there is no clear evidence that the molecular markers seen in the juice of sporadic cancer patients are also seen in patients who develop pancreatic cancer as a result of FPC. At present molecular analysis is used by the EUROPAC study group to determine the frequency of imaging.

Screening studies

There are now three groups (Johns Hopkins, the University of Washington and EUROPAC) that have pilot secondary screening programmes. The most recent of which is that proposed by EUROPAC which is outlined in Fig. 3. Participants

are recruited to the EUROPAC registry after consulting a clinical geneticist, at the time of recruitment they are advised that a pilot screening programme is available. Participants are then seen in an outpatient clinic by a consultant pancreatologist. If the participant is over 40 and belongs to a confirmed FPC kindred the possibility of screening will be raised; the limitations of the existing technologies are discussed and the potential risks of the screening modalities are explained, particularly post-ERCP pancreatitis and the risk of radiation exposure. The clinician and the participant then decide what elements of the screening programme are appropriate. The full screening programme includes a baseline analysis involving measurement of fasting glucose and CA19-9, with imaging by CT scan and EUS. Where appropriate ERCP is offered for juice analysis rather than imaging. The baseline results will lead to the participant entering either a "standard" or a "close" surveillance pathway. A deciding factor is the presence or absence of pancreatic juice DNA abnormalities. If patients undergo pancreatic juice analysis and there are no DNA changes detected, they enter the standard surveillance cycle, where serum tests, imaging and the juice analysis are repeated on a three yearly staggered basis. Patients that do not have juice analysis are entered onto the close surveillance pathway. This consists of annual follow up, serum tests and imaging. In almost all FPC patients, as they have healthy pancreatic tissue, the screening modality of choice is EUS. If the baseline imaging confirms significant fibrosis (e.g. chronic pancreatitis) CT is used in preference. To date 39 FPC patients have been screened. The programme has yet to discover its first cancer.

The University of Washington group aims to identify patients with histologically confirmed PanIN 3 lesions or very early pancreatic cancers before they progress to incurable disease. Baseline EUS is performed 10 years prior to the earliest age of onset of pancreatic cancer in that family. If the EUS is normal, the patient is offered a repeat EUS in one year. If the EUS indicates an abnormality unrelated to pancreatitis, an ERCP is offered after a discussion of risk and benefit. Patients with an abnormal EUS and a normal ERCP are followed up by EUS in one year. Patients with an abnormal EUS and ERCP are given the option of continuing with surveillance until a mass forms or obtaining a tissue diagnosis. Histology is obtained via laparoscopic resection of the pancreatic tail as needle biopsies would be inadequate to exclude the presence of PanINs. Out of a cohort of 75 patients, 15 high risk patients with abnormal EUS and ERCP have undergone surgery. Twelve patients had a total pancreatectomy, the remaining three had a partial pancreatectomy or "tailectomy" and chose to continue with surveillance. Of the 15 patients operated on so far, five had PanIN 2 lesions and ten had PanIN 3's. None of the pancreata resected were normal or had pancreatic cancer. In the remaining 60 patients that were being followed with annual imaging one patient developed an unresectable pancreatic cancer (personal communication from Dr T. Brentnall, Washington University).

The Johns Hopkins group aims to identify early pancreatic masses when the lesion is either precancerous or a resectable malignancy. The methods used are baseline EUS and CT with imaging repeated at 12 months. High risk individuals (n=78) were either recruited on the basis of FPC (n=72) or Peutz-

-Jeghers syndrome (n=6). All participants had an EUS; 67 had images that were considered abnormal, 17 of which were consistent with neoplasia. In patients where EUS detected an abnormality, FNA was performed on the pancreatic head, body, and tail. Spiral CT scanning was performed on the 67 high risk patients with an abnormal EUS and 65 accepted the offer of an ERCP, of which 64 were successful. Although ERCP did identify abnormalities (cysts, saccules, dilated ducts or other signs of pancreatitis), the Hopkins group have expressed the opinion that the benefits of ERCP imaging do not justify the risk of post-ERCP pancreatitis; 5 of the 64 participants where the pancreatic duct was cannulated developed pancreatitis as a result of ERCP. There were eight participants with pathologically confirmed neoplasms, 7 participants underwent a subtotal pancreatectomy as a result of screening. None of the participants undergoing surgery as part of the screening programme had confirmed adenocarcinoma, although IPMN and PanIN lesions were common. One participant had a cyst on CT but developed metastatic pancreatic cancer in the interval before returning to clinic [101].

Cost Effectiveness

Risk and benefit cannot only be considered in terms of patient survival and risk of maleficence; cost implications cannot be ignored. Papers have discussed the cost of cancer screening in HP and Peutz-Jeghers syndrome (PJS). Screening of hereditary pancreatitis patients was considered to be prohibitively expensive; it was calculated that it would cost \$164,285 per pancreatic cancer detected [77]. In PJS the cost per life saved was estimated at just \$50,000, which is economically viable, but only if all other causes of cancer death in PJS could be eliminated. With existing levels of cancer risk in this syndrome, the cost of screening would rise to a prohibitive \$297,000. This cost model also assumed use of molecular analysis to phase screening; without this added element costs would rise even further to \$373,000 [103]. Potentially, FPC costs would be much lower as the proportion of the screened population that would be expected to develop pancreatic cancer would be much higher. Estimation is complex, but with the use of molecular analysis, the figure would be at, or below, \$50,000 per life saved. A better idea of the true cost will be possible once the results from the pilot screening programmes have matured.

Recent advances and future challenges

The most exciting and contentious topic to emerge relating to FPC in recent months is the discovery of a Palladin mutation in Family X. The data were published late in 2006 and initially looked as if they were a definitive breakthrough. However, subsequent work has suggested that Palladin may not be the FPC gene. Work by the EUROPAC/FaPaCa [57] study groups and the NFPT [58] has shown that the 4q32-34 locus (the site of the Palladin gene) is unlikely to account for a significant proportion of families and the Palladin mutation has not been identified in other FPC kindreds [59,60].

Another contentious issue is anticipation. Rigorous testing suggests that this is a genuine phenomenon, although detractors may still question whether it is due to a statistical artefact [9]. Inclusion of anticipation in models used for linkage analysis may allow successful application of linkage or association studies in the hunt for FPC mutations.

The greatest clinical benefit that research can provide to members of FPC families would be a viable screening programme. Pilot studies have highlighted many ethical and management dilemmas. Hopefully, in the near future, they will begin to provide the solutions. Once this has been achieved, there may be scope for introducing screening in other high risk groups such as late onset diabetics. Demand for screening will surely grow as high risk individuals become more aware of their risk, but until issues of efficacy and safety have been resolved, secondary screening should only proceed in specialist centres. The process requires a collaborative approach between groups developing new screening modalities and those carrying out pilot trials.

Conclusions

Familial pancreatic cancer is a genuine syndrome leading to autosomal dominant inheritance of a predisposition to pancreatic cancer. Individuals from these families recognise they are at high risk and demand screening; but in most cases the lack of a known disease mutation makes identification of those at greatest risk difficult and the lack of a proven screening protocol limits our ability to help family members. Despite the challenges, progress is being made in many directions. It is likely that the FPC gene will soon be identified and in parallel, new screening modalities are being developed and applied in pilot studies. If risk can be adequately defined, screening studies offer the hope, not just of a greater understanding of pancreatic tumorigenesis, but the potential for early diagnosis and cure in high risk groups.

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