Determinants of risk and disease progression in complex pancreatic disorders

Thesis submitted in accordance with the requirements of the University of Liverpool for the degree of Doctor of Medicine

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Dedication

This thesis is dedicated to Professor David William Sheel.

The only person who would have read this thesis because he wanted to, not because he had to.

You taught me to be strong and resilient as if you knew I'd need that when you had to leave us.

Declaration

The work presented in this thesis was carried out in the Institute of Translational Medicine, University of Liverpool and was undertaken while working as a research fellow at Liverpool Hepatobiliary Centre with funding from the Royal College of Surgeons of England Research Fellowship.

The material contained within this thesis has not been, nor is currently being presented wholly, or in part, for any other degree or qualification. I declare that all the work presented in this thesis has been carried out by me except where indicated below:

- Assistance with complex statistical modelling in chapter 6. The extended Cox proportional hazards analysis and the sensitivity analysis was performed by Dr Ruwanthi Kolamunnage-Dona (UoL department of biomedical statistics).
- Assistance with other complex statistical analysis relating to family index and EUROPAC modelling from Prof Bill Greenhalf

A RG Sheel

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Abstract

Title: Determinants of risk and disease progression in complex pancreatic disorders

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Introduction: Chronic pancreatitis, pancreatic neoplasms and pancreatic ductal adenocarcinoma represent a spectrum of complex disorders of the pancreas. They share a common 'early disease phase' presenting an opportunity for potential screening, earlier detection, and initiation of treatment aiming to prevent disease progression. This includes progression to PDAC. However, these conditions also pose significant diagnostic challenges.

Aims: to determine risk factors associated with disease progression in chronic pancreatitis and factors associated with an increased risk of developing PDAC, especially in those already at higher risk. Through international collaboration, establish and address challenges restricting future research and clinical trials towards earlier diagnoses. Identify methods of stratifying risk of developing PDAC to allow appropriate allocation of resources and intensiveness of screening to those at the greatest risk of PDAC.

Methods: Pancreas specific clinical databases and disease registries were reviewed, refined, and analysed with view to Identifying potentially relevant risk factors, exploring and describe relationships. Complex mathematical modelling was applied to these clinical datasets to allow further risk stratification of high-risk populations for developing PDAC. A modified Delphi process was adopted to a novel international consensus guideline process from conception through to publication.

Results: Every aspect of this work, including the process of international guideline development, has highlighted the critical role that risk factors play in the diagnosis, the natural history of a condition, and disease progression. This is demonstrated in the CP specific work where we described the resolution of early CP changes at EUS following the withdrawal of risk factor exposure. When analysing individuals with an inherited risk of PDAC, diabetes and smoking were found to be competing risk factors for PDAC development in those with hereditary pancreatitis.

The EUROPAC FPC secondary screening outcomes identified one PDAC case occurring within the top 10% tier of PDAC risk. 22 BD-IPMNs were identified with equal probability across all risk categories. No high-risk individual within EUROPAC with an individualised family risk score of <30 has ever developed PDAC.

Conclusion: Risk stratification and greater consideration of individualised risk offers the potential to optimise screening for pancreatic cancer through increasing the likelihood of early detection yield whilst mitigating the unavoidable risks associated with screening.

Chapter 1: Introduction

A disease is an entity that is distinct and measurable. The term disorder may be more appropriate in a situation where a specific disease is possible, but the clinical evidence required to make a diagnosis is lacking.

Acute pancreatitis (AP), chronic pancreatitis (CP), pancreatic neoplasms and pancreatic ductal adenocarcinoma (PDAC) represent a spectrum of complex disorders of the pancreas. Some of these disorders are difficult to detect in their early stages, evolve through various states over time and have highly variable clinical courses (1). They share multiple aetiologies and pathological features, yet these pathologies can result in very different treatment responses and patient outcomes. Pathogenic germline genetic mutations and variants play an important role in complex pancreatic diseases. AP is beyond the scope of this thesis and will not be discussed further in any detail.

Whilst these complex pancreatic disorders account for most pancreatic diseases, in terms of the general population, they are relatively uncommon. Disease registries for rare diseases are crucial in defining the natural history, evolution, risk, and outcomes of diseases. They support genetic research and establishing a highly motivated and flexible patient base for developing new treatments, diagnostics, or screening techniques.

The pancreatic diseases addressed in this thesis all share a common theme of an ‘early disease phase’ which presents an opportunity for screening and initiation of treatment aiming to prevent disease progression and even the development of pancreatic cancer. However, they also present significant diagnostic challenges, especially around early CP, CP, and alternate diagnoses.

Progressing understanding of these complex disorders requires a paradigm shift in our approach with new focus on disease mechanisms rather than symptoms. There is a need to further stratify those individuals at risk and for further development of complex disease models.

Chapter 2: Aims

2.1 Aims

1. Determine the clinical, genetic, environmental factors that influence disease progression and activity in patients with CP.
2. Develop international consensus on current areas of controversy relating to CP and early CP- to make progress towards a definition and diagnostic criteria for Early CP and facilitate future research and clinical trials.
3. To further stratify PDAC risk in high risk kindreds to further enrich the screening population making secondary screening for PDAC more efficient and effective.

2.2 Hypotheses

1. Recent improvements in the understanding of complex pancreatic disorders and disease processes (Including CP) will result in the realisation that some patients have previously been misdiagnosed.
2. Chronic pancreatitis is understood to be a progressive disease therefore, patients with suspected or early chronic pancreatitis will develop clear evidence of the disease over time.
3. Molecular biomarkers can be used alongside clinical features, demographics and lifestyle characteristics (e.g. smoking) to predict disease progression and activity in patients with CP
4. There are subpopulations within high risk kindreds that are at the highest risk of developing PDAC. Restricting screening to/ targeting screening at these highest risk individuals will improve screening yield.

The aims of this thesis will be achieved through several objectives.

2.3 Objectives

1. To complete a full review of the Liverpool CP patient cohort and evaluate the evidence upon which a diagnosis of chronic pancreatitis had previously been made, with the benefit of clinical follow-up and review of clinical and imaging criteria; including the application of EUS based scoring systems in the diagnosis of early chronic pancreatitis.
2. Identifying risk factors for progression from early CP / minimal change CP at EUS features to definite chronic pancreatitis.
3. Describe the relationship between smoking, diabetes and PDAC in HP.
4. Demonstrate the importance of strict adherence to PDAC screening protocols in high-risk groups.
5. Assess if familial risk correlates with a higher incidence of PDAC precursor lesions (IPMNs) in our FPC kindreds.
6. Identifying a subpopulation of ‘the highest risk individuals’ from FPC kindreds at increased risk of PDAC.

Chapter 3: Chronic Pancreatitis

3.1 Introduction

3.1.1 The definition of chronic pancreatitis

Traditionally definitions of CP have focused on what we now understand to be end stage disease characteristics. For example, Chronic pancreatitis (CP) is characterised by continuous parenchymal inflammation which causes progressive fibrosis of the pancreas leading to structural changes of varying extent including loss of exocrine and endocrine parenchyma, calcification, and pancreatic duct obstruction, amongst others. These structural changes may result in further sequelae such as endocrine and exocrine insufficiency, biliary obstruction, gastric outlet obstruction, venous occlusion, pancreatic pseudocysts or fistulas and other significant complications.

There have been numerous historical recommendations for how one should define CP. Table 1 provides a short historic summary of the outcomes from seven of the most prominent expert consensus group meetings and workshops which have occurred over the past five decades.

Table 1. A summary of published definitions of chronic pancreatitis

Date	Expert consensus group	Summary	Limitations
1963, 1984 and 1988	The Marseille conferences (2, 3)	Initial efforts towards a consensus definition of CP, incorporating morphologic, functional and clinical criteria. Distinction was made between acute pancreatitis (characterised by complete resolution of symptoms) and permanent histological and clinical changes associated with chronic pancreatitis. Focus on end staged CP.	Lack of accepted criteria for 'irreversible morphological change'. Classification of recurrent AP lacked clarity. Limited knowledge of complex role of genetic and environmental risk factors.
1983	The Cambridge Classification of pancreatic severity (4)	A meeting of independent experts proposing improvements on the Marseille classification with recent advances in abdominal imaging techniques including CT, MRI and ERCP. CP definition based on morphology of the pancreas from ERCP features. CP defined as "a continuing inflammatory disease of the pancreas, characterized by irreversible morphological change, and typically causing pain and/or permanent loss of function."	Limited knowledge of complex role of genetic and environmental risk factors. Reliance on structural parenchymal changes related to fibrosis. However, structural/functional/clinical features are not a surrogate for each other.
1991-1994	The Japanese Pancreas Society (JPS) (5, 6)	JPS meetings to define CP; "a chronic clinical disorder, pathologically characterized by the loss of exocrine and endocrine pancreatic parenchyma, irregular fibrosis, cellular infiltration, and ductal abnormalities" Progressive nature of CP disease process acknowledged. Recognises an early pathogenic disease process that cannot be currently defined with existing morphologic methods. Recommends that treatment should be initiated in early stages	Role of genetic and environmental risk factors unknown.

1996	The Zürich Workshop (7)	A workshop to develop a clinical classification for CP of alcohol related aetiology. CP is a "classic disease without a clinically valid, generally recognized definition." Recognises an early pathogenic disease process that cannot be currently defined with existing morphologic methods.	Focus on alcohol induced CP. Limited recommendations as the environmental effects of tobacco and genetic risk factors were unknown
1999	The North American Pancreatitis Study Group (NAPSG)	Modifications of the Cambridge classification for use in the North American Pancreatitis Study II (NAPS2). CP is "syndrome of destructive, inflammatory conditions that encompasses the many sequelae of long-standing pancreatic injury". The definition aimed to be inclusive of all CP variants and allow classification of patients according to aetiology, features or outcomes. Introduction of the Sentinel Acute Pancreatitis Event [SAPE] model. Recognises an early pathogenic disease process that cannot be currently defined with existing morphologic methods.	
2013	German Clinical Practice Guidelines (8)	The multinational working group definition: "Chronic pancreatitis is a disease of the pancreas in which recurrent inflammatory episodes result in replacement of pancreatic parenchyma by fibrous connective tissue. This fibrotic reorganisation of the pancreas leads to a progressive exocrine and endocrine pancreatic insufficiency. In addition, characteristic complications arise, such as pseudocysts, pancreatic duct obstructions, duodenal obstruction, vascular complications, obstruction of the bile ducts, malnutrition and pain syndrome. Pain presents as the main symptom of patients with chronic pancreatitis. Chronic pancreatitis is a risk factor for pancreatic carcinoma. Chronic pancreatitis significantly reduces the quality of life and the life expectancy of affected patients."	Very descriptive definition of CP. Excludes atrophy and minimal change from the CP spectrum of disease.
2014	American Pancreatic Association (APA) Practice guidelines (9)	Closely related to the Cambridge classification. Recognises an early pathogenic disease process that cannot be currently defined with existing morphologic methods. Attempts histopathological classification of CP including; "atrophy and fibrosis of the exocrine tissue with or without chronic inflammation".	
2016	International consensus Proposal (10)	The mechanistic definition of CP recognises the complex nature of the disease, encourages rational approaches to early diagnosis, disease classification and prognosis. Focuses on a pathogenic disease process which can be predicted before pathological changes occur. Thus, allowing early initiation of preventative measures.	

		“Chronic pancreatitis is a pathologic fibro-inflammatory syndrome of the pancreas in individuals with genetic, environmental and/or other risk factors who develop persistent pathologic responses to parenchymal injury or stress.’ In addition, “Common features of established and advanced CP include pancreatic atrophy, fibrosis, pain syndromes, duct distortion and strictures, calcifications, pancreatic exocrine dysfunction, pancreatic endocrine dysfunction and dysplasia.”	

3.1.2 The challenges of defining CP

Many of the previous guidelines and consensus meetings listed above merely summarise the current approach to defining, diagnosing, and managing CP. Historically this was heavily weighted towards advanced disease with evidence of irreversible end staged radiological features (calcifications for example) steatorrhea, diabetes mellitus, and pathological changes required for diagnosis.

The definition of CP itself precluded identification of the disease in an early state and therefore early aetiological based treatments were inconceivable. Any advancements in this fields would require the disease to be redefined based around a framework which utilises informative biomarkers to allow early diagnosis, early classification, and improved personalised treatments to give a better prognosis.

A new Mechanistic Definition of CP was proposed to define the mechanism of disease and the typical characteristics of established disease (10). The two-part Mechanistic Definition is, “Chronic pancreatitis is a pathologic fibro-inflammatory syndrome of the pancreas in individuals with genetic, environmental and/or other risk factors who develop persistent pathologic responses to parenchymal injury or stress.” and “Common features of established and advanced CP include pancreatic atrophy, fibrosis, pain syndromes, duct distortion and strictures, calcifications, pancreatic exocrine dysfunction, pancreatic endocrine dysfunction and dysplasia.”

This new Mechanistic Definition is not reliant on radiology to define the disease. It recognises that CP is a complex disease, risk factors are considered separate from markers of disease activity and disease end points. This is a step in the right direction towards achieving early diagnosis, classification, prognosis, and individualised therapy.

3.1.3 The epidemiology of CP

There are limited epidemiological studies covering the incidence, prevalence, gender distribution and natural history of chronic pancreatitis disease

progression due to the large variety in diagnostic criteria for CP employed globally. Annual incidence rates are estimated around 4 per 100,000 in the UK (11) and the USA (12) up to 13.4 / 1000,000 in Finland (13).

More recently there has been a rise in the incidence of CP. The reasons for this are not clear especially when most cases of CP in the western world are attributed to alcohol consumption and tobacco smoking and these practices are declining. The increase in incidence therefore may be due to improvements in our understanding of CP, improvements in cross sectional imaging and other diagnostic modalities, along with improved availability and access. The prevalence of CP is reported around the 30-50 per 100,000 population level (12, 14-17), however, this may be a significant underestimate due to the challenges of disease definition, diagnostic criteria, patient compliance and long survival (median survival time from diagnosis is around 15-20 years (17)).

Traditionally males have been reported up-to five times more likely to develop CP however, more recent data from the US The North American Pancreatitis Study (NAPS and NAPS2) studies have suggested that the prevalence of CP in women may have previously been underestimated (18) due to the differing common aetiological factors between the two genders. Toxic aetiologies such as alcohol and tobacco smoking are far more common in males (16, 19), whereas females are more likely to develop CP due to obstructive pathologies or idiopathic pancreatitis. The NAPS2 study showed that CP was more common amongst black patients and was often more severe. Further work is needed here to account for socioeconomic differences in the USA.

Furthermore, the risk of developing pancreatic cancer is increased 10-fold in patients with sporadic CP and up to 70-fold in patients with hereditary pancreatitis (HP) (with proven PRSS1 mutation) (20-22).

3.1.4 Aetiology and risk factors

There are three main classifications for the aetiology of CP, namely the traditional classification, the M-ANNHEIM system which classifies risk factors

for CP into 7 categories (23), and the TIGAR-O system (24). The latter has been updated in the TIGAR-O V2 system (25)) which is the most commonly used as it was developed with the assumption that an individuals' risk of developing CP is multifactorial. Groupings here are by risk modifiers rather than aetiologies and categorised as toxic-metabolic, idiopathic, genetic, autoimmune, recurrent and/or severe acute pancreatitis-associated, and obstructive (24). Toxic-metabolic causes include alcohol (26-29), smoking (30, 31), hypercalcemia (32-34), hyperlipidaemia (35, 36), chronic renal failure (37, 38), medications, and toxins (39). Although there is strong evidence that SARS Co-V2 is associated with acute pancreatitis in male patients with a pre-existing metabolic syndrome (Acute Pancreatitis Metabolic Syndrome), its association with chronic pancreatitis is yet to be determined (40).

The classifications are summarised in table 2. The TIGAR-O system was used in the international consensus guidelines for chronic pancreatitis process as described in chapter 5.

Table 2. Published classification systems for the aetiology of chronic pancreatitis

Classification for aetiology of CP	
Traditional	
Alcohol	
Idiopathic	
Hereditary	
Obstructive	
Hyperlipidaemia	
TIGAR-O (24)	
T	Toxic-metabolic; alcohol, tobacco, hypercalcaemia, hyperlipidaemia, chronic renal failure, medications, toxins
I	Idiopathic
G	Genetic mutations including PRSS1, CFTR, SPINK-1, others
A	Autoimmune; Isolated, systemic
R	Recurrent and severe acute pancreatitis associated CP
O	Obstructive; Pancreas divisum, sphincter of oddi disorders, obstruction / structuring of the pancreatic duct
M-ANNHEIM (23)	
M	Multiple risk factors including;
A	Alcohol consumption. (Excessive consumption (>80 g/day). Increased consumption (20–80 g/day). Low-Moderate consumption (<20 g/day))
N	Nicotine consumption (In cigarette smokers: description of nicotine consumption by pack-years)
N	Nutritional factors. Nutrition (e.g., high caloric proportion of fat and protein). Hyperlipidemia
H	Hereditary factors. Hereditary pancreatitis (defined according to Whitcomb)(41). Familial pancreatitis (defined according to Whitcomb)(41). Early-onset idiopathic pancreatitis. Late-onset idiopathic pancreatitis. Tropical pancreatitis.
E	Efferent duct factors. Pancreas divisum. Annular pancreas and other congenital abnormalities of the pancreas. Pancreatic duct obstruction (e.g., tumours). Posttraumatic pancreatic duct scars. Sphincter of Odd dysfunction.
I	Immunological Factors. Autoimmune pancreatitis. Sjögren syndrome-associated chronic pancreatitis. Inflammatory bowel disease-associated chronic pancreatitis. Chronic pancreatitis with autoimmune diseases (e.g., primary sclerosing cholangitis, primary biliary cirrhosis).
M	Miscellaneous and rare metabolic factors. Hypercalcaemia and hyperparathyroidism. Chronic renal failure. Drugs. Toxins

Geographical variations in CP aetiology are notable. The leading cause of CP in the industrialised sectors (the west and Japan) is excessive alcohol consumption which accounts for up to 70-90% of cases (42-44). Whereas Asian populations have a high prevalence (20-125 per 100,000 individuals) of 'tropical CP' (45); this is a rather historical term and in reality causation is most likely an interplay between environmental factors and mutations with serine protesase inhibitor kazal - type1 protein, encoded by the SPINK gene (45-50).

The commonly accepted threshold for 'alcohol excess' is around 20g/day to 80g/day (23, 42, 51), with the NAPS2 study further clarifying this to 60-80g/day (52). A large systematic review and meta-analysis performed by Irving et al, demonstrated an exponential dose-response relationship between average amount of alcohol consumed and incidence of pancreatitis. Individuals consuming 36 g of alcohol per day had a relative risk (RR) of 1.2 (95% CI 1.2-1.3) compared with non-drinkers, while those consuming 96 g per day had a four-fold increased risk of pancreatitis (RR 4.2, 95% CI, 3.1-5.7) (26). The exact pathogenic mechanisms are unclear, but alcohol metabolites cause direct toxic effects on pancreatic acinar cells by interfering with protective mechanisms against endoplasmic reticulum stress and also lowering the threshold for the initiation of acute pancreatitis. There is no evidence to suggest there is a temporal relationship between length of time of excess alcohol consumption and development of CP, although some studies have shown that cessation of alcohol consumption after an acute pancreatitis episode can reduce the risk of progressing to CP (53).

Tobacco smoking has previously been under recognised as a risk factor for the development of CP however, there is now strong evidence to support it as an independent risk factor with a dose-dependent relationship (52, 54, 55), a disease modifier with adverse synergistic effects when combined with alcohol excess, and a factor that accelerates disease progression and mortality (56). The pooled risk estimates for current smokers compared to lifelong non-smokers were 2.8 (95% CI:1.8-4.2) overall and 2.5 (95% CI:1.3-4.6) when data were adjusted for alcohol consumption.

3.1.5 The pathogenesis of chronic pancreatitis

Chronic pancreatitis is a pathologic fibro-inflammatory syndrome of the pancreas characterised by progressive fibrotic destruction of the pancreatic parenchyma following recurrent episodes of acute pancreatitis and chronic inflammation. CP develops in Individuals with genetic, environmental and/or other risk factors who develop persistent pathologic responses to parenchymal injury or stress (10). “This fibrotic reorganisation of the pancreas leads to a progressive exocrine and endocrine pancreatic insufficiency. In addition, characteristic complications arise, such as pseudocysts, pancreatic duct obstructions, duodenal obstruction, vascular complications, obstruction of the bile ducts, malnutrition, and pain syndrome. Pain presents as the main symptom of patients with chronic pancreatitis. Chronic pancreatitis is a risk factor for pancreatic carcinoma. Chronic pancreatitis significantly reduces the quality of life and the life expectancy of affected patients” (57).

The pathogenesis of pain in CP is poorly understood as it is a multifactorial disease with a complex clinical picture. Genetic variations (discussed below) play a clear role in the pathogenesis of CP but not only increasing susceptibility of developing CP but also contributing to the variability seen in the clinical course of the disease. Environmental factors also influence disease onset and progression.

There is growing evidence to support the key role that pancreatic stellate cells (PSC) play in the development of fibrosis of the pancreas resulting in deposition of extracellular matrix in the areas of acinar and ductal cell injury and/or necrosis (58, 59). Progressive fibrosis leads to irreversible architectural changes eventually causing endocrine and exocrine failure (malnutrition and diabetes mellitus) and the classic radiological appearances of advanced or end staged CP.

Numerous mechanisms for the development of CP have been proposed. The five main mechanisms are summarised below. The trypsin dependant pathway was first described in 1896 and premature trypsin activation has subsequently been found in all models of pancreatitis as well as human samples of pancreatitis (60). However, demonstration of premature trypsinogen activation in the pancreas does not necessarily establish causality for CP.

3.1.5.1 Necrosis-fibrosis

CP develops following repeated episodes of severe acute pancreatitis with inflammatory cells and pancreatic stellate cells replacing areas of necrosis and injury with fibrotic tissue (61). A history of severe necrotising pancreatitis is however uncommon in patients with CP.

3.1.5.2 Direct toxic-metabolic effect

This mechanism proposes there is a direct toxic effect on acinar cells from the toxic metabolites of environmental risk factors (such tobacco and ethanol consumption) which change intracellular metabolism leading to pancreatic lipid accumulation (62). Epidemiological studies have however shown that most people exposed to the risk factors do not develop CP.

3.1.5.3 Oxidative stress due to free radicals

By-products of hepatic oxidase activity damage the pancreas through chronic reflux of bile into the pancreatic duct (63). This theory has been better established in the development of AP (64).

3.1.5.4 Ductal dysfunction

Dysfunction in the pancreatic duct can lead to inadequate flushing of enzymes into the duodenum and formation of protein plugs which cause ductal blockages and upstream obstruction and back pressure on the acinar cells (65). Pancreatic stones may form which can also cause ulceration, scarring, and stricturing of the pancreatic duct. It is unclear if these events are causative or a consequence of CP.

3.1.5.5 Sentinel acute pancreatitis event (SAPE) model of pancreatic disease

The SAPE model of pancreatic disease is a two-hit model (66) designed to test the hypothesis that the development of CP requires a triggering (but resolving) event that results in hypersensitivity of the pancreas to RAP and further injury. The inflammatory process is triggered by an initial pancreatic injury (episode of AP) and there is infiltration of inflammatory macrophages and activation of pancreatic stellate cells. Acute pancreatitis is typically self-limiting with complete recovery of the pancreatic tissue and resumption of normal pancreatic function. However, patients with particular risk factors and disease modifiers are at higher risk of developing recurrent

pancreatitis and on-going stressors drive inflammatory responses including the activation of local immune cells causing continuous inflammation, fibrosis, atrophy, and other features of CP. A subset of these will develop the cardinal features of CP (67). A SAPE rat model has been developed where multiple episodes of AP resulted in fibrosis (68). Further evidence to support the SAPE model came from two separate studies showing after an initial AP attack, RAP occurred most in individuals with alcohol excess, genetic mutations, and to a lesser extent smoking (69, 70).

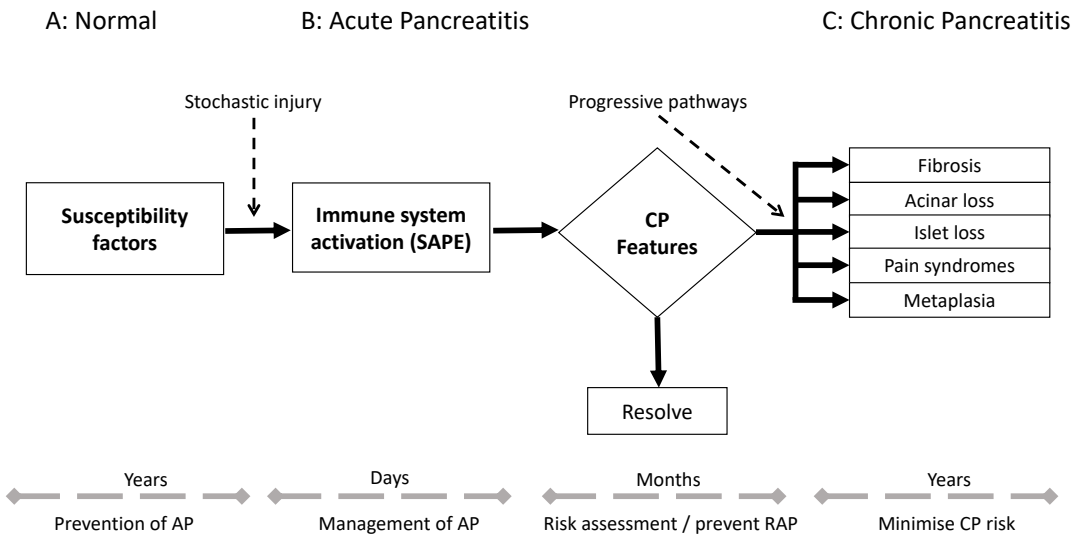


Figure 1. The SAPE hypothesis model adapted with permission from DC Whitcomb(1)

3.1.6 The genetics of chronic pancreatitis

CP is a complex disease with multiple genetic risk factors and disease modifiers. There have been significant genetic discoveries over the last 25 years, improving our understanding of genetic susceptibility to CP. Genetic mutations and variations with a strong association to CP include PRSS1 (cationic trypsinogen) (41), SPINK1 (serine protease inhibitor kazal-type 1) (71, 72), and CFTR (cystic fibrosis transmembrane conductance regulator) (73, 74) and, to a lesser extent, CTRC (chymotrypsin C) (75, 76) and CASR (calcium-sensing receptor) (77, 78). PRSS1, SPINK1 and CTRC mutations cause pancreatitis via the trypsin dependant pathway. They target acinar cells by promoting trypsinogen activation and by impairing protective trypsinogen degradation and/or trypsin inhibition. Figure 2 summarises the trypsin dependant

pathway. Alternative pancreatitis pathological pathways have emerged, notably the mutation induced protein misfolding mechanism(79).

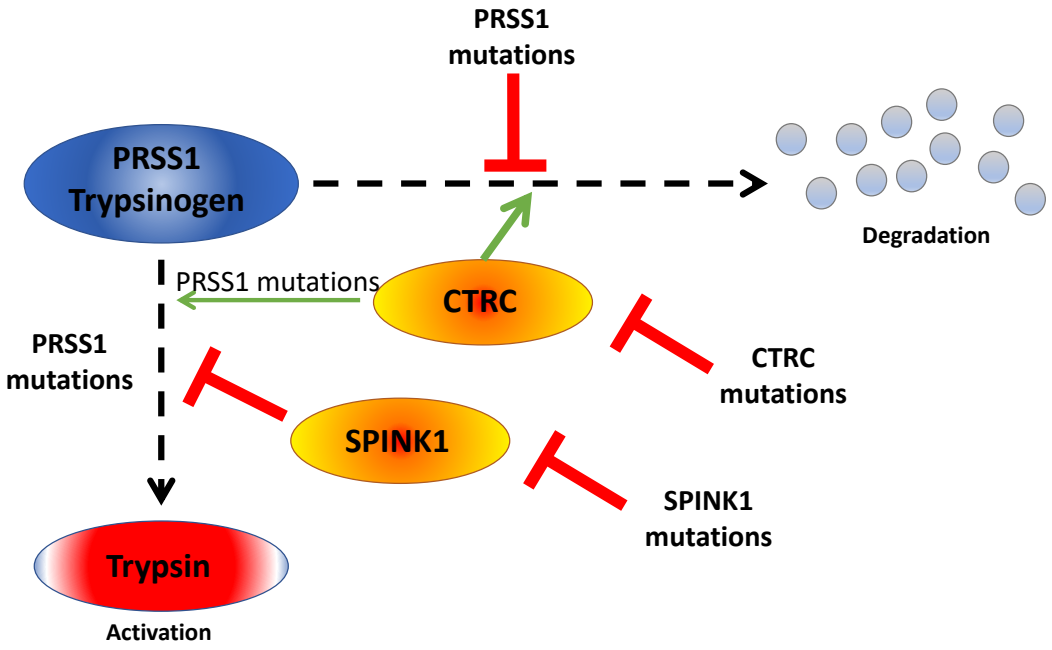


Figure 2. The Trypsin dependant pathway in chronic pancreatitis. Adapted from Hegyi et al (72) (80)

3.1.6.1 Hereditary pancreatitis and PRSS1

Hereditary pancreatitis is a rare form of chronic pancreatitis accounting for approximately 1% of all CP cases (19). It is an autosomal dominant (AD) condition characterised by recurrent attacks of acute pancreatitis starting in childhood with typically earlier progression to clinical features of CP including fibrosis, chronic abdominal pain, pancreatic exocrine failure (malnutrition) and pancreatic endocrine failure (diabetes mellitus) than is usually seen in patients with CP from other causes. HP also carries an approximate 40 % lifetime risk of developing PDAC (21).

HP is caused by a mutation in the cationic trypsinogen gene (PRSS1) leading to increased stability of the trypsin product causing autodigestion. The HP gene was first localised to the q arm of chromosome 7 by Claude Ferec in 1996 (81) and later that year Whitcomb et al identified the p.R122H mutation in the cationic trypsinogen (PRSS1) gene as a cause of HP(41). PRSS1 gain of function mutations lead to autodigestion by activating the enzyme cascade in the acinar cell and causes recurrent

acute pancreatitis by altering the export of trypsinogen, initiate self-activation of trypsin, and/or increase the caldecrin activation of trypsinogen (82). The mutations generally cluster around the 2 calcium-binding pockets that are critical in regulating trypsinogen activation and trypsin inactivation. This discovery has intensified investigation of the role of premature activation of trypsin in the acinar cell as critical in initiating pancreatic injury. Mechanisms of action for different PRSS1 variants have been described. R122H and to a lesser extent N29I mutations increase trypsinogen autoactivation by inhibition of CTRC-dependent trypsinogen degradation whereas A16V (+/- N29I) increases CTRC-dependent stimulation of trypsinogen autoactivation.

Subsequently, at least 25 other pathogenic PRSS1 mutations have been reported. The three most common PRSS1 mutation variants are p.R122H (accounting for at least 50%), p.N29I and arguably A16V. Penetrance is high, in the region of 70-80%. However, each variant displays differing penetrance levels and phenotypes (81) (see Figure 3). The p.R122H, p.N29I variants share the most similar phenotype, whereas A16V mutations can provide a highly variable picture (22).

Some kindreds display a typical AD pattern of inheritance however, no disease-causing mutation has yet been identified.

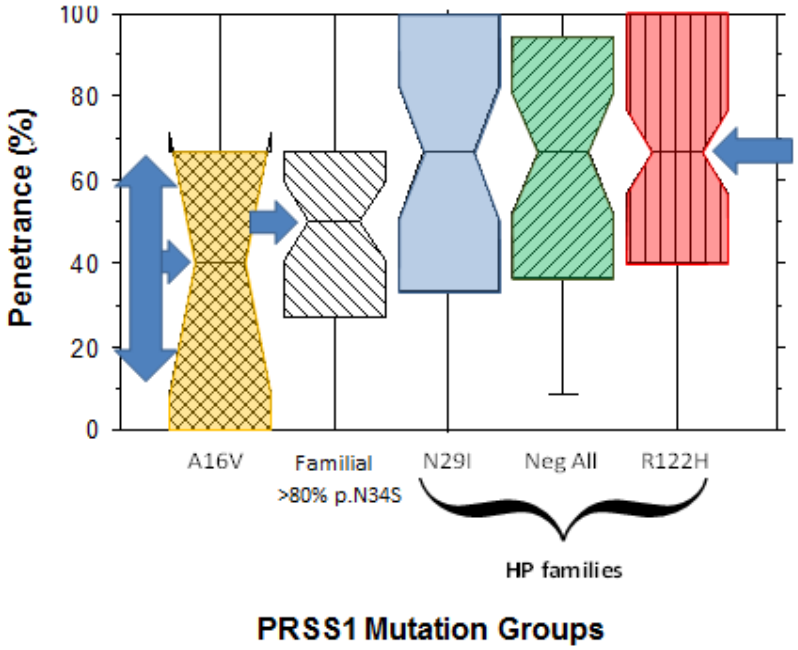


Figure 3. Disease penetrance with differing PRSS1 variants adapted from Grocock et al (22)

3.1.6.2 Idiopathic pancreatitis

About 10% of all CP cases are diagnosed as idiopathic (83). Genetic mutations associated with idiopathic pancreatitis but with no clear pattern of inheritance include *SPINK1* (serine protease inhibitor, Kazal type 1), and *CFTR* (cystic fibrosis transmembrane conductance regulator) (74, 82).

3.1.6.3 Serine protease inhibitor, Kazal-type 1

SPINK1, also known as pancreatic secretory trypsin inhibitor PSTI) is a protein secreted by the pancreatic acinar cells that may inhibit trypsin by delaying trypsinogen autoactivation. *SPINK1* reacts with a newly generated trypsin molecule during autoactivation, and this trypsin is then unavailable to catalyse further trypsinogen activation. It is thought that *SPINK1* activity accounts for the inhibition of around 20% of trypsin activity until reserves are depleted (84). There have been over 30 variants of *SPINK1* reported however, the p.N34s variant is the most frequently encountered. Multiple studies have shown an association between the presence of a *SPINK1* p.N34s mutation and the development of acute and/or chronic pancreatitis (85). Witt et al. reported the p.N34s variant was present in 18/85 (21%) children with idiopathic pancreatitis (82) however, heterozygous *SPINK1* p.N34s mutations were also found

in 1-2% of the general population leading to the conclusion that carrying a SPINK1 p.N34s mutation alone is not sufficient to explain the development of CP and therefore is a disease modifier rather than a direct causative risk factor (86).

3.1.6.4 Cystic fibrosis transmembrane conductance regulator

Cystic fibrosis transmembrane conductance regulator (*CFTR*) is most associated with the autosomal recessive disorder, cystic fibrosis (CF), a disease caused by high chloride output from secretory cells, predominately in the lungs and the pancreas. CFTR variants are very common in the general population (1:25) however, variants would need to be inherited from both parents typically (Homozygous *CFTR) to* cause the classic 'Cystic fibrosis' phenotype. Some heterozygous variants can cause less severe phenotypes who can develop CP, male infertility, and chronic sinusitis (87). CFTR is vital for the proper functioning of the pancreatic duct. It is a chloride-bicarbonate anion channel expressed on the ductal epithelium which secretes bicarbonate to flush pancreatic enzymes secreted from the acinar cells into the pancreatic ducts and subsequently the duodenum. Mutations thus increase the risk of pancreatitis due to impaired duct flushing. CFTR mutations may have a synergistic relationship with SPINK1 mutations as the presence of both results in a higher risk of developing acute pancreatitis, recurrent acute pancreatitis, and CP (88, 89).

3.1.6.5 Chymotrypsin C (CTRC)

Chymotrypsin C (CTRC) codes for a protein that promotes the degradation of trypsin and has been shown to be associated with idiopathic and alcoholic chronic pancreatitis (76). Loss-of-function mutations in CTRC increase the risk for chronic pancreatitis by compromising protective trypsinogen degradation.

3.1.7 Imaging modalities in the diagnosis of CP and other pancreatic diseases

3.1.7.1 Computer Tomography (CT)

CT is relatively non-invasive and cheap, with the added advantage of being and readily available. Abdominal CT is widely accepted as the initial investigation of choice when assessing a patient for a potential diagnosis of CP (9). The classic radiological features of established CP (glandular changes, main pancreatic duct dilatation and stricturing, atrophy, and pseudocyst formation as well as involvement of adjacent

blood vessels and organs) are evident on CT imaging (9, 90). This modality also provides information of the presence of any CP related complications and disease progression including any concerning features for the development of PDAC. CT can also exclude other differential diagnoses which have a similar symptomatology to CP. The sensitivity and specificity of CT in CP has been reported as 75% and 91%, respectively (91).

Up to date CT imaging of the pancreas is also vitally important with any interventional endoscopy or operative planning (90). CT cannot exclude or confirm a diagnosis of early CP where morphological changes may be subtle or not detectable on CT. The discrete nature of the morphological changes in early CP remains one of the greatest challenges in diagnosis.

3.1.7.2 Magnetic Resonance Imaging (MRI)

Magnetic Resonance Cholangiopancreatography (MRCP) is becoming increasingly popular in the investigation and diagnosis of CP particularly in patients with equivocal CT images but a convincing history for early CP for example. MRCP is most useful in providing information about the pancreatic ductal architecture specifically strictures, cystic lesions, and ductal dilatation. MRI is reported to have a slightly higher sensitivity and specificity for the diagnosis of CP compared to CT (78% and 96% respectively) (91). Currently the main advantage of MRI / MRCP in the investigation of CP is its ability to differentiate between CP from cystic lesions (90). Previously secretin was used to stimulate pancreatic juice and allow better imaging of the ductal system, however this product has been withdrawn by the Medicines and Healthcare products Regulatory Agency (MHRA) due to provocation of acute on chronic pancreatitis in these patients.

3.1.7.3 Endoscopic Ultrasound (EUS)

EUS has been used in the assessment of CP patients for over two decades and provides accurate information on pancreatic pathology allowing easy identification of a normal pancreas compared to one which is heavily diseased however, this information can also be obtained with standard cross-sectional imaging. EUS has an increasing role in evaluation of CP and its associated complications. Most recently discussions have focused on the role of EUS in the assessment and diagnosis of early

and/or mild CP. EUS has the greatest potential to identify the subtle parenchymal and ductal changes associated with early CP however, data are inconsistent and unreliable as these changes can be found in other conditions that are distinct from CP. Some of these subtle features have been used in the development of certain diagnostic criteria for CP and early CP although the ideal number of EUS features needed to make an accurate diagnosis remains unknown.

The controversies surrounding the role of EUS in the diagnosis of early CP and the relevant diagnostic criteria are discussed in more detail below.

3.1.7.4 Endoscopic Retrograde Cholangiopancreatography (ERCP)

Historically Endoscopic Retrograde Cholangiopancreatography (ERCP) was the 'gold standard' test for the diagnosis of CP and the Cambridge classification system was developed based on ERCP findings along with the presence of calcifications. ERCP provided highly accurate assessments of pancreatic ductal morphology and has been the test all other modalities have been compared against (92). Diagnostic ERCP in CP has now been largely abandoned due to the risk of post ERCP acute pancreatitis.

3.1.7.5 Other investigations in CP

The role of pancreatic function testing in the diagnosis of CP are limited. Pancreatic exocrine and endocrine failure are both relatively late features of the natural history of CP disease progression, and neither are pathognomonic for CP. Steatorrhoea is only clinically evident when 90% of the pancreas has been damaged (93). A clinical system of history of steatorrhea and serial glycated haemoglobin (HbA1c) is adopted by most.

3.1.8 Classification and diagnostic criterion in CP

When discussing criteria for CP, diagnostic criteria and classification criteria are sometime used interchangeably. For clarification, in this piece of work diagnostic criteria refers to the collection of signs and symptoms used to diagnose and treat a disease. These criteria are generally broad as they need to reflect heterogenicity within diseases. Classification criteria are the standardised definitions of a condition used to create a uniform group of patients for clinical research. Due to the lack of gold

standards in CP and early CP, any criteria (classification or diagnostic) are difficult to establish.

3.1.8.1 Classification criteria

The aim of a classification criteria opposed to a diagnostic criterion is to use standardised definitions to create homogenous groups of patients to facilitate clinical studies and trials. Validated classification criteria are vital to clinical research as they allow direct comparison between study results. Typically, classification criteria have a high specificity with a lower sensitivity resulting in more false negatives, potential misclassification and the exclusion of individuals who may have the condition in question. Therefore, meeting classification criteria is not equivalent to achieving a diagnosis.

The majority of classification systems for CP follow from the Cambridge 1984 classification (4) and/or the Zürich workshop recommendations (7) (See table 1) and use pancreatic morphology criteria. Cases not meeting 'definite CP' imaging criteria but with a strong suspicion of CP were classed as 'probable CP'. The M-ANNHEIM classification system is strongly influenced by the Zürich definitions of CP apart from the apparent 'downgrading' of patients with classical CP symptoms but no morphological changes evident on pancreatic imaging and normal pancreatic function from "probable CP" to "Borderline CP" (23). The 2014 American Pancreatic Association (APA) Practice Guidelines in Chronic Pancreatitis gave no definition for CP and aired on the side of caution recommending less reliance on classifying and diagnosing patients with CP given the current lack of understanding of the diseases' natural history (9). They suggested classification of patients as "Definitive", "Probable", and "Insufficient" based on imaging studies or histology.

3.1.8.2 Diagnostic criteria

There are several diagnostic criteria systems for CP published however, at present there is no single internationally accepted and validated diagnostic criteria for CP. The most referenced criteria include the 'standard criteria' (94), the Rosemont criteria (95) and the 'Japanese criteria' (96). These criterions and their key features are described in table 3. In summary, the standard and the Rosemont criteria focus purely on EUS findings, whilst the Japanese criteria considers imaging, histological findings alongside

clinical features. The standard criteria are the most commonly used whereas the Rosemont criteria is thought to be more stringent but less specific. A retrospective cohort study scoring EUS examinations with both the standard criteria and the Rosemont criteria found the conventional standard criteria correctly identified more cases of CP (97).

3.1.8.2.1 Standard classification

The most used scoring system is the standard classification (94, 98) uses nine equally weighted criteria based on four parenchymal and five ductal features. A threshold of 3-5 criteria is considered strong support for the diagnosis of CP.

3.1.8.2.2 Rosemont classification

The EUS Rosemont classification was developed at a consensus conference with 32 internationally recognised endosonographers in attendance as a weighted, standardised method to improve EUS CP scoring. It includes many features of the Cambridge classification, which was based on ERCP, but also provides information on the parenchyma (4, 95). Criteria were divided into major A and major B based on perceived predictive accuracy. According to these criteria, CP can be diagnosed in the presence of (a) 1 major A feature + $\geq$ 3 minor features, (b) 1 major A feature + major B feature, and (c) 2 major A features. This system however is based entirely on expert opinion and has yet to be validated.

3.1.8.2.3 The Japanese clinical diagnostic criteria system

The Japanese criteria uses the standard criteria for parenchymal and ductal changes and allows for ‘definite CP’ diagnosis if there are also key imaging findings and / or characteristic histological findings (96).

There are six main diagnostic criteria: (1) characteristic imaging findings such as parenchymal calcification, ductal calculus, ductal morphological changes, and pseudocyst, (2) characteristic histological findings of loss of exocrine parenchyma with irregular predominantly interlobular fibrosis, (3) repeated upper abdominal pain, (4) elevated pancreatic enzyme levels in serum or urine, (5) reduced pancreatic exocrine function, and (6) continuous heavy drinking > 80g/day (10 units a day), using the Ammann criteria for a diagnosis of chronic pancreatitis secondary to alcohol (7). A

diagnosis of definite chronic pancreatitis requires criteria 1 and/or 2 to be met. Early chronic pancreatitis requires three of four ‘abnormal’ parenchymal findings (i-iv, see Table 3) plus at least three out of clinical criteria 3-6. Possible chronic pancreatitis is diagnosed when patients have at least three of criteria 3-6, in the absence of either criterion 1 or 2, following the exclusion of other pancreatic diseases.

Table 3. Diagnostic criteria for chronic pancreatitis

Features of Chronic Pancreatitis	Standard Criteria (94)	Japanese Diagnostic Criteria (18)	Rosemont criteria (95)
Parenchymal	Hyperechoic foci	i. Hyperechoic foci without shadowing	Major A: Hyperechoic foci (>2 mm in length/width with shadowing) Minor: Hyperechoic foci (>2 mm in length/width, without shadowing)
	Hyperechoic strands	ii. Stranding	Minor: Hyperechoic strands (≥3 mm in at least 2 different directions with respect to the imaged plane)
	Lobularity	iii. Lobularity without honey combing iv. Lobularity with honey combing	Major B: Lobularity (≥3 contiguous lobules = 'honeycombing') Minor: Lobularity (>5 mm, non-contiguous lobules)
	(Pseudo) Cysts	(Pseudo) Cysts	Minor: (Pseudo) Cyst (anechoic, round/elliptical with or without septations)
Ductal	Main duct dilatation		Minor: Dilated duct (≥3.5 mm in body or >1.5 mm in tail)
	Duct irregularity		Minor: Irregular duct contour (uneven or irregular outline and ectatic course)
	Hyperechoic margins	Hyperechoic main pancreatic duct margin	Minor: Hyperechoic duct wall (echogenic, distinct structure >50% of entire main pancreatic duct in the body and tail)
	Visible side branches	Dilated side branches	Minor: Dilated side branch (>3 tubular anechoic structures each measuring ≥1 mm in width, budding from the main pancreatic duct)
	Intraductal stones		Major A: Duct calculi (echogenic structure[s] within the main pancreatic duct with acoustic shadowing)
Diagnosis	Standard Criteria (94)	Japanese Criteria (18)	Rosemont criteria (95)

	High probability for chronic pancreatitis: 5 to 9 criteria	Definite chronic pancreatitis: criteria 1 and/or 2 (1) characteristic imaging findings (calcifications, calculus, ductal morphological changes), (2) characteristic histological findings of loss of exocrine parenchyma with irregular predominantly interlobular fibrosis	Consistent with chronic pancreatitis: 1 Major A feature + ≥3 minor features 1 Major A feature + major B feature 2 Major A features
		Early chronic pancreatitis: EUS image findings of early chronic pancreatitis (three of i-iv) plus more than 2 items among criteria 3-6 (3) repeated upper abdominal pain (4) elevated pancreatic enzyme levels (serum or urine) (5) reduced pancreatic exocrine function (6) continuous heavy drinking > 80g/day (10 units a day, Ammann criteria (37)	Suggestive of chronic pancreatitis: Major A + <3 minor Major B + ≥3 minor ≥5 minor, no major
	Indeterminate for chronic pancreatitis: 3-4 criteria	Possible chronic pancreatitis: More than 2 of criteria 3-6, in the absence of criteria 1 or 2, with exclusion of other pancreatic diseases	Indeterminate for chronic pancreatitis: Major B alone or with + < 3 minor 3 to 4 minor features, no major
	Normal or low probability of chronic pancreatitis: 0-2 criteria		Normal: <3 minor, no major

3.1.9 Severity scoring

There have been several scoring systems published to score the severity of CP. However, the current roles and utility of these systems are somewhat restricted given the lack of consensus surrounding early CP and the lack of validated diagnostic criteria. The Cambridge classification for severity has been previous discussed and used ERCP ductal changes to indicate severity (4). The system has evolved and been

adapted over the years to be applicable to more contemporary imaging modalities. The ABC system suggested by Büchler et al. has a three staged classification which considers clinical criteria including pain, RAP, pancreatic failure and local complications, along with ductal and parenchymal features seen on imaging (99). The Rosemont criteria have been discussed prior along with M-ANNHEIM classification, which also attempts to evaluate the severity of inflammation through symptoms and the response to certain therapies. The Chronic Pancreatitis Prognosis Score (COPPS) is a complex points-based system developed to describe the severity of CP. It considers a variety of clinical factors including frequency and length of hospital admission with CP related episodes (100).

3.1.10 Early Chronic pancreatitis

The concept of early chronic pancreatitis is well acknowledged but beyond this there is little consensus. There are no established, accepted, or validated definitions of diagnostic criteria for early CP and as discussed above, even defining CP itself has not been without challenges.

Traditionally our understanding and definitions of CP have been based on end staged/advanced CP features. More recent evidence has shown however, that many of these features are not unique to CP and are also present in the pancreata of individuals without CP. There is now a growing appreciation for the wide spectrum of stages and features of CP. Beyond the classical features of advanced or end staged CP, there is no consensus on what constitutes a different stage of CP, or what the classification of other CP stages are even.

By accepting the concept of early CP, we accept that there is a potential window of opportunity for earlier diagnosis and earlier initiation of treatment in the disease process which may slow, halt, or even reverse the changes seen in CP.

EUS is thought to be the imaging modality of most relevance when looking for subtle pancreatic parenchymal and ductal changes associated with early CP. EUS diagnostic criteria were thought to provide the best opportunities for diagnosis early CP however all EUS based criteria will inevitably be hampered by challenges relating to reliability, inter and intra-observer variability and interpreter bias (101). Some of the subtle pancreatic changes identified on EUS and previously thought to indicate early CP have

now been shown to be normal variations of pancreatic appearance. Some changes can also fluctuate over time and are influenced by other factors such as age and smoking (102, 103). In addition, there are no validated standardised diagnostic criteria and the ideal threshold number of EUS criteria to help establish a diagnosis of early CP remains unknown.

3.1.11 The symptoms of CP

Symptomatology of CP is intimately related to the development of CP related complications. The long-term morphological sequelae of chronic inflammation, fibrosis, and loss of parenchymal architecture results in failure of the exocrine and endocrine pancreas. Pancreatic exocrine insufficiency is a reduction in the secretion of digestive enzymes and bicarbonate from the pancreas affecting the normal ability to digest nutrients. This results in the development of malnutrition, manifesting as weight loss, steatorrhea, abdominal bloating, muscle weakness, osteoporosis, neurological abnormalities, and visual defects. Endocrine insufficiency is due to decreased hormones including insulin being secreted from the beta cells in the islets of Langerhans and causes type 3c pancreatogenic diabetes mellitus. This typically occurs later in the natural history of the disease.

3.1.12 The management of CP and its sequalae

At present all management and treatment modalities in CP are focused on symptomatic control and treatment of CP related complications in end staged or advanced CP. There are no effective treatments for early CP.

3.1.12.1 Pain

Pain is typically the first symptom that patients present with, is often the most distressing, and the most challenging to manage. Classically this is epigastric radiating through to the back. Some patients have incidental findings of advanced CP changes on radiology but are completely pain free. Several studies have failed to explain the discrepancy between pancreatic morphology and severity of pain. The mechanisms for pain in CP are complex and how pain is experienced is multimodal. Pain is directly related to pancreatic / peripancreatic complications and abnormalities in the peripheral

/central nervous system. Chronic pain is extremely challenging to treat and manage effectively.

Analgesic relief follows the principles of the World Health Organisation ‘analgesic ladder’ with a step wise introduction of analgesic agent potency. Most patients do not gain adequate symptomatic relief from simple analgesics alone and require opiate based medications, which are associated with their own management challenges including opiate dependence and opiate toxicity. There is also a role for adjuvant analgesic agents including pregabalin (104).

Pain caused from direct pressure effects within the pancreatic ductal system from pancreatic stones or ductal strictures may respond better to invasive therapies (interventional endoscopy / surgery) to relieve the ductal hypertension. The role for enzyme supplementation and antioxidant therapy in pain management in CP is unclear. Pancreatic enzyme replacement with high protease content may aid in reducing pancreatic secretions and therefore lowering ductal pressures (105). Oxidative stress is an accepted mechanism contributing to the development of CP related inflammation. One study has shown some analgesic benefit when pregabalin was taken with antioxidants in management recurrence CP related pain following surgical or endoscopic intervention (106).

There is increasing evidence to support earlier surgical intervention to provide long term pain relief in chronic pancreatitis. Traditionally surgery was seen as a last resort however several cohort studies and the recent ESCAPE randomised control trial advocate early surgery within 2-3 years of diagnosis to achieve better pain associated outcomes (107, 108).

3.1.12.2 Pancreatic failure and enzyme replacement

Failure of the exocrine pancreas results in inadequate production of pancreatic digestive enzymes leading to maldigestion, malabsorption, malnutrition. These can manifest as abdominal bloating, steatorrhoea, weight loss etc and are easily treated with pancreatic enzyme replacement therapy (PERT). PERT is taken as a supplement during food ingestion and the dosing of can be modified depending on the size and fat content of meals and snacks to achieve optimum symptomatic control (109, 110). A

typical starting regime would advise around 75,000 units with main meals and 50,000 units with snacks. A proton pump inhibitor should also be prescribed regularly.

Type 3c diabetes mellitus is seen in advanced chronic pancreatitis. Diabetes management is highly personalised, can be refractory to treatment (especially in the case of brittle diabetes) and needs to consider the development of diabetes related complications including hypertension, retinopathy, and neuropathy, therefore should be managed by specialists. Treatment is often with long-acting insulin and as required short acting insulin supplements.

3.1.12.3 Other Sequalae

Other sequelae include ductal and parenchymal calcifications, ductal strictures, inflammatory masses, pseudocysts, biliary and duodenal obstruction, pancreatic fistulae, and pancreatic ascites (14, 17, 24, 83, 111). Vascular complications include porto-mesenteric venous compression or occlusion, extra-hepatic portal hypertension, splenic-portal-thrombosis, venous collateralisation, and pseudo-aneurysm (111-115). Finally, there is also the increased risk of developing pancreatic ductal adenocarcinoma.

3.1.12.4 Interventional endoscopy and surgery

Longitudinal studies show that a large proportion (up to 40-75%) of CP patients require some form of endoscopic and/or surgical intervention in their lifetime, most commonly (in 80-90%) for intractable pain *(42, 107, 116, 117)*. Current standard practice employs an ‘endoscopy first approach’ where following failure of opiate analgesia, some patients are offered endoscopic pancreatic ductal stone removal, ESWL, and/or stenting of ductal strictures. Surgery is reserved for resistant cases where all other treatment options have been exhausted and has been seen as the ‘nuclear option’ due to the morbidity and mortality risks associated with pancreatic surgery.

Duodenum- preserving pancreatic head resection (DPPHR) notably the Beger, Frey and Berne procedures are effective for pancreatic head dominant disease, providing decompression of the duodenum, main pancreatic duct, and intra-pancreatic bile duct (118, 119).

There remains a role for total pancreatectomy in a highly select group of patients with end staged CP affecting the entire pancreas, intractable pain, and pre-existing endocrine failure. (120-122).

A randomised controlled trial (RCT) has previously shown that for patients with end stage CP surgical treatment to be more effective that endoscopic management in mid to long term pain relief (123, 124). Several observational studies have suggested that surgical intervention earlier in the disease course may also help to provide better pain control whilst also preserving pancreatic function (125-128). The recent Dutch Pancreatitis Study Group conducted a multicentred RCT (ESCAPE trial) investigated whether early surgical intervention is more effective than the endoscopy-first approach for improving clinical outcomes. They found overall reduced pain scores when integrated over 18 months in the early surgery group however, further work here is required (108).

Total pancreatectomy with islet autotransplantation (TPIAT) has been used since the late 1970's in the treatment of chronic pancreatitis aiming to relieve pain and prevent the development of brittle diabetes through islet autotransplantation (129). The role of TPIAT in the management of CP remains controversial and there is geographical variation in its use. Whilst there is general agreement that in the right patient TPIAT has the potential to improve overall quality of life, there is no clear consensus on who the 'right' patient is. Furthermore, TPIAT is most successful in preventing pain and brittle DM when performed in the early stages of CP to maximise the yield of functioning islet cells however, with no agreed definition or diagnostic criteria for early CP there is the possibility that patients misdiagnosed with early CP may be inappropriately offered surgery which carries mortality risk, the need for life long nutritional supplementation, exocrine replacement, and diabetes management.

3.1.1 Chronic pancreatitis and pancreatic ductal adenocarcinoma

The link between pancreatic cancer and chronic pancreatitis is complex. Patients with chronic pancreatitis are at increased risk of pancreatic malignancy (15, 130) with an overall relative risk of 13 (131, 132). As discussed above, individuals with HP PRSS1 mutation and those who smoke or consume excess alcohol are at particular risk. CP and PDAC are both differential diagnoses for most pancreatic diseases and they can

co-exist making their differentiation a particular diagnostic challenge (133). In the most complex cases, a definitive diagnosis can only be achieved after surgical resection. Here, the morbidity and mortality risk for surgery is accepted when compared to the risk of overlooking a probable pancreatic cancer.

3.2 A new, accurate definition for CP

The traditional approach towards the investigation and management of CP is not working. Overall, CP has been largely ignored at worst and misdiagnosed or misunderstood at best. All treatments are focused on end stage disease when changes are already irreversible and there are no improvements in prognosis to be made. The challenges around CP are centred on a) the definition of the disease, b) the diagnosis and c) aetiologies:

A) The initial definition of CP, developed during the 1989 Marseille-Rome classification, was a descriptive-pathologic definition for CP with the primary goal of distinguishing CP from other diseases with similar features of pancreatic inflammation (2). This definition required advanced disease features and pathology as the primary state and thus failed to define the essence of the underlying disorder or the unique pathogenic processes involved.

B) Pancreatic tissue is not routinely available for histopathological analysis, so the 1984 Cambridge conference developed clinically diagnostic criteria at ERCP for the diagnosis of CP. This required irreversible pancreatic fibrosis as the key defining feature. Furthermore, recent histopathological international consensus guidelines have agreed that histology is not the gold standard for diagnosis of CP and pancreatic fibrosis is not pathognemonic for CP, and may not be evident at all in cases of early CP.

C) CP has traditionally been understood to be a disease of alcohol excess however only around 5% of alcohol dependants develop CP (134). There has been an increase in the number of idiopathic CP cases in parallel to improvements in imaging quality and accessibility. Primary aetiology of CP cannot be the direct cause.

The first breakthrough came with the discovery of hereditary pancreatitis where gain-of-function mutations in the cationic trypsinogen gene (*PRSS1*) resulted in. an autosomal dominant, high-penetrance form of RAP/CP. This discovery highlighted the role genetics played in the development of CP. Further studies into HP lead to key observations which inspired Whitcomb to develop the SAPE model (discussed above, Figure 3). PRSS1 mutation carries were normal until they had an initial episode of AP, an acute pancreatitis attack appeared to sensitise the pancreas to further episodes (RAP) and RAP drove progression to CP.

Once we accept the SAPE model, then we must consider the complex multifactorial relationships between aetiological factors and additional genetic and environmental factors which influence disease activity and progression from AP, RAP to CP. Therefore, we must also consider the need for a more accurate definition of the CP syndrome. A definition that would allow for early diagnosis and the exclusion of alternative disorders and diseases which share similar features. Whitcomb et al believe the new mechanistic definition of CP has heralded a paradigm shift in our approach to CP (10). An evolution away from viewing CP in terms of its end staged histopathological features whilst embracing new insights and disease models that provide a framework for early detection of the pathogenic pancreatitis process.

3.3 The conceptual CP model

The conceptual model of CP as depicted in Figure 4 was derived from the SAPE hypothesis model (10) as a progressive CP pathogenesis model to organise the risk, activities, and stage of the CP process. In this model, CP is the result of progression of an individual from no disease (A. at risk) to pancreatic destruction (E. End stage CP). The process is typically ‘activated’ with a “sentinel AP event” AP and RAP (135), which may not be clinically recognised or recorded. Damage to acinar and duct cells (C- D), and fibrosis (immune cells), leads to diabetes (islet cells), pain syndromes (nervous system) and dysplasia / pancreatic ductal adenocarcinoma (PDAC). The key opportunity for intervention and novel therapeutics is between stages B and D.

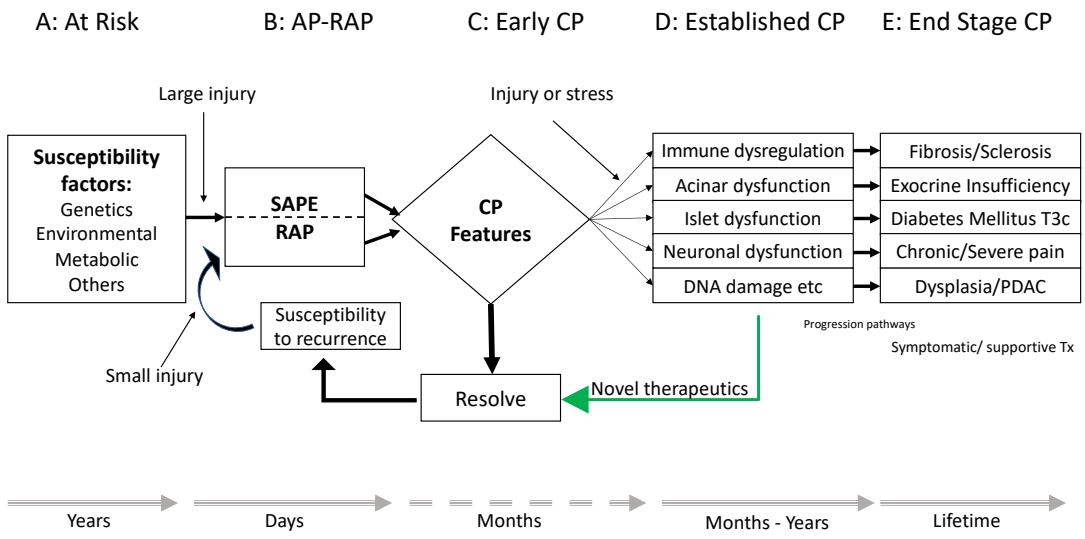


Figure 4. The conceptual model of progressive CP pathogenesis adapted with permission from DC Whitcomb (10)

Stage A: Everyone within this group has an increased risk of CP, but there is currently no disease.

Stage B: AP and RAP, both of which are well defined self-limiting acute disease processes. No individual in stage B has CP but AP and RAP are well recognised risk factors for CP.

Stage C: Individuals with one or more features of CP (e.g on imaging, pancreatic failure, abdominal pain) but do not have features of advanced CP. This group is discussed further below.

Stage D: Traditional CP accompanied with classic features such as pain and pancreatic dysfunction (although some function may be reserved).

Stage E: End stage CP associated with classical irreversible advanced features including pancreatic failure, type 3C diabetes mellitus, malnutrition, chronic pain potentially requiring surgical intervention, and neoplasia/PDAC.

3.3.1 Stage C – the black box

The ‘CP black box’ is a term coined by David Whitcomb himself as the area that provides the greatest challenge in the diagnosis and assessment of CP. It represents

the most complex part of the CP conceptual model where the complex mechanisms at play are understood the least. This 'box' includes all individuals with early signs and or symptoms of early CP. These patients will represent a spectrum of people from those with true early CP to those with a normal variation of their pancreas to those with poorly defined alternate pancreatic disorders (136). There are several clinical syndromes that are not chronic pancreatitis but share numerous overlapping clinical features. This includes disorders of chronic abdominal pain (such as the CAPS cohort discussed in this chapter), functional syndromes, and pathology in the pancreas caused by different pathological mechanisms, such as extrinsic duct obstruction, fibrosis from the desmoplastic reactions in pancreatic cancer or the pancreatopathy seen in long-standing diabetes mellitus for example(136).

3.4 The Liverpool CP database

In additional to coordinating EUROPAC (European Registry of Hereditary Pancreatitis and Familial Pancreatic Cancer), which is the largest registry of patients with HP in Europe, Liverpool also hosts one of the most extensive biobanks of clinical samples from patients with chronic pancreatitis in the UK (> 700), with peripheral blood and tissue samples from all consenting patients (See methodology below). Further, there is extensive epidemiological data, and clinical records including radiology spanning over decades available for those patients. The CP biobank and databases were merged and formally recognised with ethical approval in 2010. Prior to this data were collected as part of a generic pancreatic biobank and database.

3.4.1 Refining the diagnosis of CP

A diagnosis of chronic pancreatitis has life changing consequences and is compounded in individuals who suffer from severe chronic abdominal pain without any abnormal imaging findings to explain the symptoms (106, 137, 138). A number of patients may have been incorrectly or prematurely diagnosed with chronic pancreatitis, especially in the setting of subtle and/or non-specific imaging features. This can result in the initiation of incorrect management plans, unnecessary further invasive investigations, and at worst inappropriate radical treatments such as total pancreatectomy with islet autotransplantation (TPIAT).

As chronic pancreatitis is a progressive disease, we hypothesised that patients with suspected or early chronic pancreatitis would develop clear evidence of the disease over time (9, 10, 14, 103, 139). With evolving concepts of chronic pancreatitis against a background of inherent uncertainty surrounding the diagnosis of early disease, it was therefore necessary to review all individuals included in the CP database/biobank confirming their initial diagnosis remained consistent. Thus, ensuring the CP database remains; accurate, relevant, and fit-for-purpose in ongoing or future CP related research. We also identified a cohort of patients under clinical follow up who according to Whitcomb's conceptual model of CP would be within the 'CP black box'. These patients were also reviewed to determine whether an initial diagnosis remained consistent over time.

3.5 Aims and hypothesis

3.5.1 Hypothesis

1- Recent improvements in the understanding of CP disease processes may result in the realisation that some patients have previously been misdiagnosed as having CP.
2- CP is now understood to be a progressive disease, therefore patients with suspected or early chronic pancreatitis would develop clear evidence of the disease over time

3.5.2 Aims

1- To complete a full review of our local CP patient cohort and evaluate the evidence upon which a diagnosis of chronic pancreatitis had previously been made, with the benefit of clinical follow-up and review of clinical and imaging criteria; including the application of EUS based scoring systems in the diagnosis of early chronic pancreatitis.
2- To identify risk factors for progression from early CP / minimal change CP at EUS (MCEUS) features to definite chronic pancreatitis.
3- To explore the impact of the timing of an episode of acute pancreatitis in relation to the identification of MCEUS features.

4- As chronic pancreatitis is a progressive disease, we hypothesized that patients with suspected or early chronic pancreatitis would develop clear evidence of the disease over time.

3.6 Methods

3.6.1 The CP database and Biobank

A dedicated CP database and biobank was formally established with favourable ethical approval in 2010 (10/WN003/46). This ethical approval covered the attainment of samples of blood from patients with suspected or confirmed CP and the collection and storage of patient demographic, clinical, and genetic data in a dedicated CP database. This ethical approval expired in 2015, therefore at the earliest opportunity in this piece of work, a new ethical approval application was submitted and subsequently received a renewed favourable ethical opinion (REC Ref 16/WA/0057). This new application would allow the ongoing recruitment of consenting patients to the CP biobank and database in addition to the collection of research blood samples on any patient who had a clinical suspicion or formal diagnosis of chronic pancreatitis. The application was also modified from the previous approval to highlight the new inclusion of patient radiological data (future and historic) and radiological body composition analysis data in any research relating to CP.

3.6.2 Existing pancreas database of clinical metadata

Prior to the establishment of the CP database and biobank, there has been a well-established local 'pancreatic resectional' sample biobank in existence at the RLBUHT since 1997. Biological samples were collected from patients undergoing pancreatic surgery for any indication (PDAC, CP, AP, pNET etc). These samples included blood (stored as serum, plasma, and cell pellets) and urine collected on the morning of surgery, intra-operative collection of pancreatic juice, tru-cut biopsies of pancreatic tissue during surgery (stored immediately in liquid nitrogen at the point of collection), and histological specimens of normal and diseased sections of pancreas, bile duct and duodenum were collected with histopathological support once the specimen had been resected and moved to the histopathology department. Due to the nature of the disease process and the clinical indications for surgery, patients captured in this

biobank represent a subgroup of the CP population with the most severe / advanced disease which has progressed to the point of requiring surgical resection, or those patients which CP who have been misdiagnosed initially as having PDAC due to the complex clinical diagnostic challenges posed by CP.

To assess for potential biomarkers of progression and disease activity in CP, samples were required from patients who had varying levels of disease severity (early to advanced) and various levels of disease activity. As focus was on assessing disease severity within CP, only a small number of healthy volunteer patient samples were required to act as controls.

3.6.3 Patient screening and identification

The protocol for the identification of potential and/or confirmed CP patients involved systematic screening of the patient lists for all of the four pancreato-biliary surgical outpatient department (SOPD) clinics which were held weekly. Screening was performed by the clinical research fellow (AS for this work, 2011-2013 MJ) and supported by the CP research nurse and the CP database manager.

SOPD patient lists were cross referenced with the existing CP database to identify all patients who had previously been recruited to ensure a) duplication in recruitment was avoided and b) appropriate patient follow-up data collection sheets were made available for completion if patients consented.

For new patient referrals or patients who had previous been seen in the pancreas SOPD not been previously recruited to the CP biobank, all available clinical information including referral letters, clinic letters, existing patient notes, biochemical and genetic investigations, histological result from any previous pancreatic procedures and previous radiological imaging studies were reviewed by the clinical fellow to establish if the patient was eligible to be included. Cases with any uncertainty were discussed with the parent consultant.

3.6.4 Imaging and pathology

Radiological imaging modalities frequently used in the investigation of patients with suspected or confirmed CP include CT, EUS, MRI, MRCP, and less frequently secretin stimulated MRCP. The most commonly performed imaging was CT and Figure 5 gives an example of the variety of radiological appearances seen across the Liverpool CP

population. Radiological images were reviewed directly when available. Real time recording of EUS procedures are not routinely performed and on average only 2-4 key still images are saved by the endoscopists, making retrospective review unreliable. Therefore, formal review of the written EUS reports were relied upon. Endoscopic retrograde cholangio-pancreatography (ERCP) was not performed for diagnostic purposes in the RLUBHT.

A diagnosis of chronic pancreatitis was accepted at any time point during the patients clinical follow up. This included the development of diagnostic features of chronic pancreatitis from previously normal or MCEUS features indicative of possible early chronic pancreatitis.

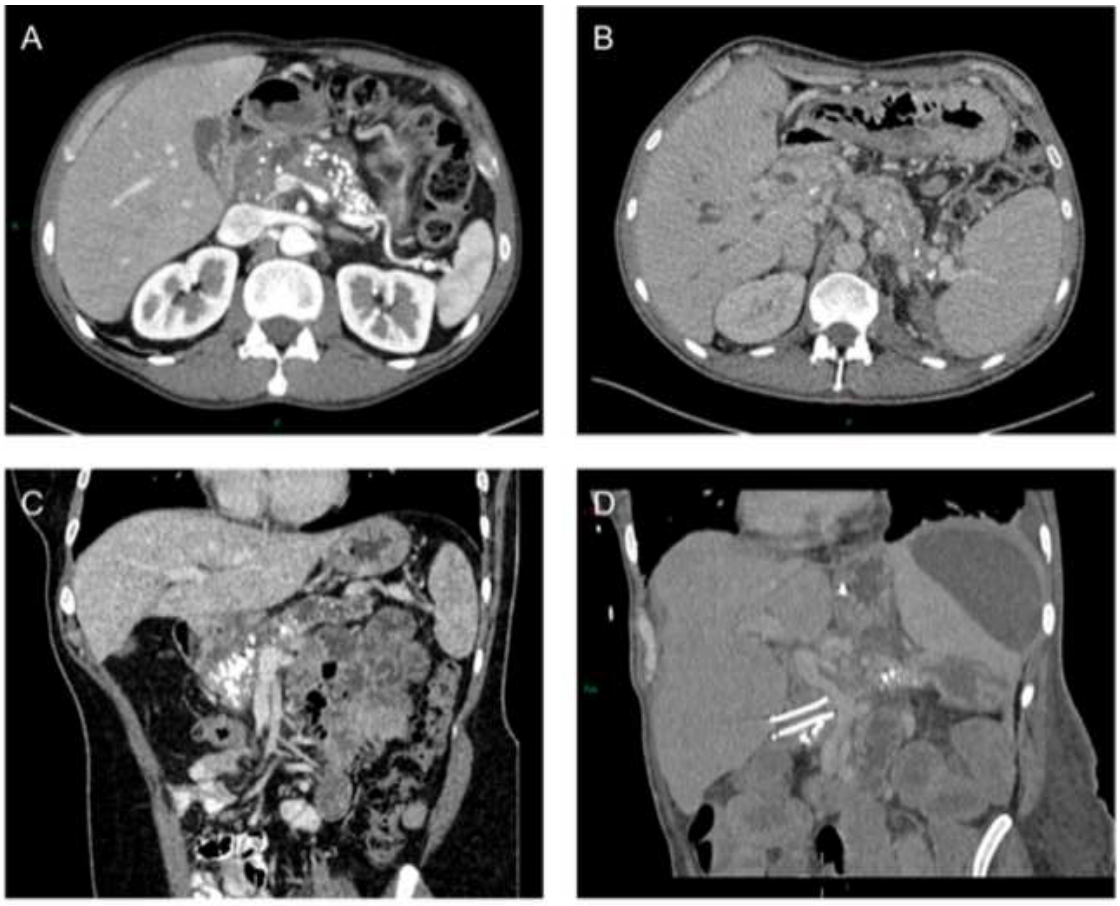


Figure 5. Example of the variation in radiological appearances in four different patients included in the CP database.

(A) Parenchymal atrophy and main pancreatic duct dilatation with diffuse parenchymal and ductal calculi. Stenosis of the splenic vein and varices. (B) Hepatic portal and splenic vein thrombosis, and splenomegaly, with splenic and gastric vein varices. Extra-hepatic bile duct occlusion with intra-hepatic duct dilatation and a previous left nephrectomy. (C) Pancreatic parenchymal atrophy with diffuse pancreatic parenchymal and ductal calculi and upstream main pancreatic duct dilatation. Splenic and gastric vein varices. (D) Duodeno-pseudocyst covered stent, non-occlusive hepatic vein thrombus and splenic vein occlusion, upper abdominal varices, splenomegaly with inferior pole infarction and large subcapsular collection. Left sided pleural effusion.

3.6.5 Patient recruitment

Once identified, potentially eligible patients were approached by a member of the CP research team in accordance with ethical approval. They were provided with the official patient information sheet (PIS), given sufficient time to process the information and ask questions, and if in agreement formally consented. Following the completion of written consent form, the patient was then invited to provide a voluntary research blood sample. Blood was collected, processed (within 2 hours) and stored as serum, plasma, and cell pellets according to the standard operating procedures (SOPS) of the GCLP facility (GCLPTSS055/2 and GCLPTSS040/1).

3.6.6 Confirmation of CP diagnosis

For the purposes of this section/body of work, the Japanese criteria was used (96). Unlike the standard and the Rosemont criteria which focus purely on EUS findings, the Japanese criteria considers imaging, histological findings alongside clinical features. A definite diagnosis of CP was confirmed in the presence of characteristic histological appearances at histopathological assessment (in the Liverpool cohort this is almost exclusively from surgical resection specimens) including loss of pancreatic exocrine parenchyma(or acinar tissue) and irregular interlobular fibrosis, and duct changes (96, 140), and/or radiological imaging with characteristic features including; calcifications, calculus, and ductal morphological changes (90).

3.7 Results

A total of 1,247 patients over the age of 18 years were referred to the Liverpool Pancreatic team in the study period with a suspected diagnosis of CP from January 2003 – November 2016. From these, a total of 807 patients including 527 men and 280 women with a median (IQR) age of 57 (48-66) years were formally diagnosed with CP and had sufficient clinical records available to be reviewed as part of this work. The outcome of the clinical case note review is summarised in the CONSORT diagram below (Figure 6).

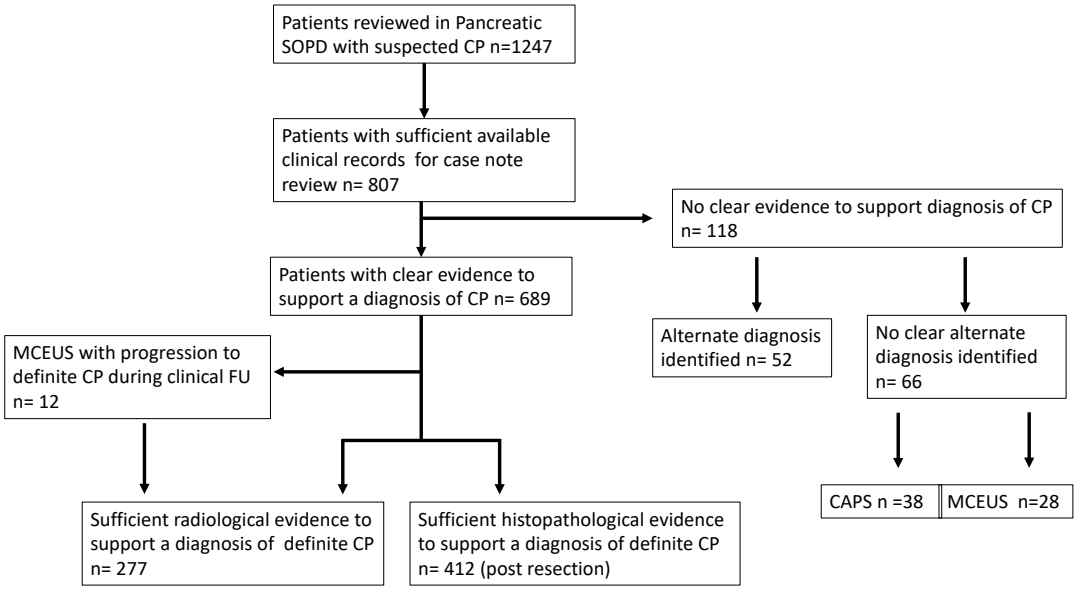


Figure 6. Consort diagram highlighting of the cohort of patients who had their diagnosis of chronic pancreatitis reviewed.

CAPS (chronic abdominal pain syndrome). MCEUS (minimal change for CP findings at EUS)

Most patients (689 / 85.4%) within the studied Liverpool CP cohort had sufficient evidence for histological and/or radiological definite CP. The remaining 118 patients (14.6%) had no clear evidence to support a diagnosis of CP. Of these 118 patients; 52 had obvious alternative diagnoses. The remaining 66 patients all had clinical symptoms of abdominal pain but on full review of their notes did not have sufficient evidence to support a diagnosis of CP and in addition and no clear alternative diagnoses. This interesting subgroup are discussed in more detail below.

Patients who had their CP diagnosis reviewed as part of this study were then sub classified according to Whitcomb's conceptual model of CP as previously outlined in Figure 4. The 66 patients with clinical features of CP including abdominal pain and some with known susceptibility factors would be examples of stage C or the "CP Black box". Sub classification and description of the Liverpool CP cohort based on the conceptual CP model is demonstrated in Figure 7.

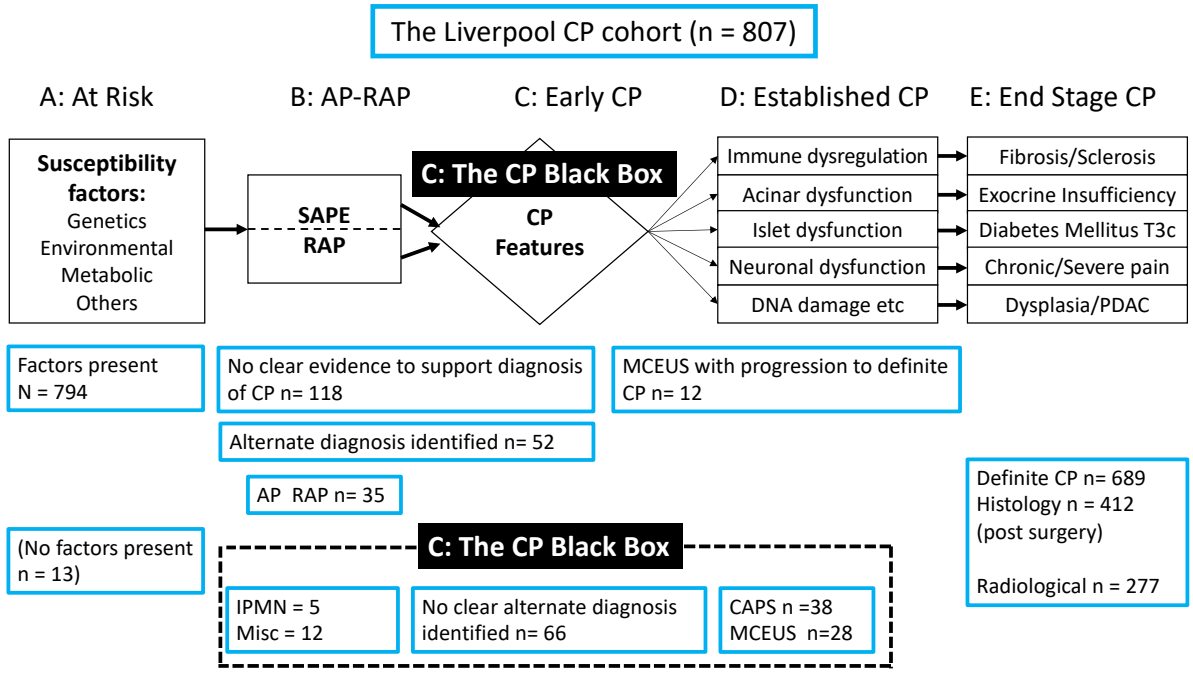


Figure 7. The Liverpool CP cohort subclassified according to Whitcomb's conceptual model of CP.

3.7.1 Patients without definite evidence of CP and no alternate diagnosis (Stage C or "the CP black box").

Following the detailed review of the Liverpool CP patient cohort, an interesting subgroup of patients emerged. These patients (n=66) had previously been diagnosed as having 'CP'. According to Whitcomb's conceptual CP model these patients would appear in stage C and fall into the "CP black box". All had clinical symptoms of abdominal pain with a median (IQR) duration of symptoms of nine (4-14) years however, no patient had any clear evidence to support a diagnosis of CP and there was no clear alternative diagnosis.

This subgroup was analysed in further detail. To proceed pragmatically, these patients have been further subclassified into 2 groups. Those with symptoms of chronic abdominal pain and normal pancreatic imaging were placed in the chronic abdominal pain syndrome (CAPS) group (n=38), and those with chronic abdominal pain in the presence of 'minimal change CP' features at initial EUS that either resolved or did not progress over the study follow up period were placed in the MCEUS group (n=28). This subgroup was felt to be highly relevant due to the importance currently placed on

the role of minimal change CP EUS findings in current clinical practice and literature relating to the diagnosis of early CP. The clinical features of the patients in these two groups are summarised in table 4.

Table 4. Clinical features of patients reclassified into the chronic abdominal pain syndrome group and the initial finding minimal change chronic pancreatitis group.

Clinical Variables	Chronic Abdominal Pain Syndrome N= 38	Initial finding of MCEUS N=28	*P value	Total N=66
Gender (Male: Female)	19:19	17:11	0.388	36:30
Age at first symptoms Median (IQR) years	43 (31-47.5)	38 (24.25-44.25)	0.143	40 (30-46)
Duration of pain Median (IQR) years	10 (4.25-14.75)	9 (4.0-12.75)	0.460	9 (4-14)
Clinical exocrine insufficiency	6 (15.8%)	5 (17.9%)	0.539	11 (16.7%)
Pancreatic enzyme supplements	25 (65.8%)	15 (53.6%)	0.315	40 (60.6%)
Insulin dependent diabetes mellitus	3 (7.9%)	2 (7.1%)	0.644	5 (7.6%)
Oral hypoglycaemics for diabetes mellitus	4 (10.5%)	0 (0%)	0.102	4 (6.1%)
Consumed ≥62 units per week of alcohol for ≥1 year	13 (34.2 %)	7 (25.0%)	0.421	20 (30%)
Alcohol units consumption per week in excess drinkers Median (IQR)	160 (82-315)	112 (70-560)	0.968	116 (85-297.5)
Ever smoker	25 (65.8%)	18 (64.3%)	0.899	43 (65%)
Current smoker (%)	12 (31.6%)	6 (21.4%)	0.360	18 (27.3%)
Number of pack years in ever smokers. Median (IQR)	20 (11.25-30)	10 (6-18)	0.021	15 (10-25)
Gainful employment	6 (15.8%)	10 (35.7%)	0.062	16 (24.2%)
Regular morphine and/or other strong opiate(s)	31 (86.1)	14 (50%)	0.006	40 (60.6%)
Daily morphine or strong opiate(s)	21 (55.3%)	8 (28.6%)	0.031	29 (43.9%)
Cholecystectomy	11 (28.9)	8 (28.6%)	0.839	19 (28.8%)
One attack of acute pancreatitis	13 (34.2%)	21 (75.0%)	**0.001	34 (51.5%)
Deaths during study period	2 (5.3%)	0 (0%)	0.328	2 (3.0%)

*P values are shown without Bonferroni correction as exploratory, except where stated.
**P value with Bonferroni correction is significant, p<0.0029.

3.7.1.1 Burden of investigation

This small subset of patients clearly presented a diagnostic challenge which is reflected in the high burden of investigation. There was a total of 266 pancreas specific imaging investigations performed over a median (IQR) clinical follow-up of 4.5 (2.2 – 6.7) years with a median (IQR) of 4 (3-5) radiological investigations per patient. The breakdown of imaging modality frequency in the two subgroups is given below.

Table 5. Number of radiological investigations performed In the CAPS and MCEUS patient subgroups.

Imaging modality	CAPS	MCEUS
CT	120	60
MRCP/ssMRCP	14	8
EUS	28	36
Total	162	104

3.7.1.2 Risk factors for CP (Stage A)

Of the 807 patients reviewed from the CP database, 794 had one or more susceptibility or risk factor for the development of CP recorded. A more in-depth risk factor analysis was undertaken within the subgroup of patients classed as stage C or in the “CP black box”. This process highlights the specific challenges faced by clinicians relating to the diagnosis of CP, most specifically early CP, versus other conditions which have overlapping clinical features of CP. The risk factors present in this patient cohort and therefore analysed included alcohol excess, tobacco use, previous AP episode/s, and predisposing genetic mutations.

All patients in the combined CAPS and non-progression MCEUS group presented with clinical features of chronic pancreatitis; all presented with chronic abdominal pain, the majority n= 58 (88%) had at least one risk factor for developing CP (table 6). Thirty-two of the 38 (84.2%) patients with CAPS and 26 of the 28 (92.9%) patients with MCEUS features of CP had between one and three risk factors associated with chronic pancreatitis. The total number of risk factors are presented in table 6 below.

Table 6. The number of recognised risk factors for chronic pancreatitis identified in the population, including significant alcohol consumption, tobacco smoking, previous episode of acute pancreatitis, and altered predisposing gene(s)

Patient Group	Number of Risk Factors				
	0	**1**	**2**	**3**	**4**
Chronic Abdominal Pain Syndrome					
Number of patients N=38	6 (15.8%)	11 (28.9%)	16 (42.1%)	5 (13.2%)	0 (0%)
Total number of risk factors N=58	0 (0%)	11 (19.0%)	32 (55.2%)	15 (25.8%)	0 (0%)
Initial finding of MCEUS					
Number of patients N=28	2 (7.1%)	10 (35.7%)	6 (21.4%)	9 (32.1%)	1 (3.6%)
Total number of risk factors N=53	0 (0%)	10 (18.9%)	12 (22.6%)	27 (50.9%)	4 (7.5%)
Total number of patients N=66	8 (12.1%)	21 (31.8%)	22 (33.3%)	14 (21.2%)	1 (1.5%)
Total number of risk factors N=111	0 (0%)	21 (18.9%)	44 (39.6%)	42 (37.8%)	4 (3.6%)

There were no significant differences in the prevalence of CP risk factors between the CAPS and MCEUS groups (p = 0.102), nor in the differences in the overall number of risk factors by ordered categories (odds ratio = 1.84, 95% confidence interval = 0.75, 4.53, p-value = 0.255).

Following clinical assessment, family history and appropriate consenting, forty underwent genetic testing for alterations in SPINK-1, CFTR and, if a family history of pancreatitis consistent with autosomal dominant trait was present, we also tested for PRSS1 gene mutations (HP). Four patients had heterozygous CFTR DF508 mutation

and two patients had a heterozygous SPINK-1 N34S variant giving a relatively frequency in this study population of 10% and 5% respectively, which is consistent with the literature.

All the patients in both the CAPS and the MCEUS group had a similar number and distribution of risk factors, supporting the view that the presence or absence of one or more risk factors alone cannot be used to infer a diagnosis of early chronic pancreatitis in the absence of characteristic imaging findings or histopathology.

3.7.1.3 The importance of timing of imaging investigations following an episode of acute pancreatitis (stage B – AP - RAP)

Of the 807 patients reviewed, 34 were found to have susceptibility factors for CP and also to have had recorded episodes of AP or RAP (without definite evidence to support CP diagnosis). This group would be classified as Stage B according to the CP conceptual model. In depth analysis of the Stage C subgroup showed that 34 of the 66 (51.5%) had either reported a previous episode/s of AP, or had biochemical and/or radiological evidence of previous AP. The proportion of patients with a history of previous AP was significantly higher in the MCEUS group (21/28 (75%)) compared to the CAPS group (13/38 (34.2%)) ($p<0.001$). Table 7 demonstrates there were no differences in clinical characteristics between patients with or without a history of AP.

Of the 34 patients who had a history of AP, 30 of these had at least one EUS as part of their diagnostic work up. Fifteen of these EUS examinations were undertaken within a 12-month period of the patient suffering from an AP attack, and these patients were more likely to be reported as having MCEUS changes ($p=0.007$).

Table 8 gives a breakdown of the EUS features seen in these patients.

Table 7. Clinical characteristics comparing those with and without a history of acute pancreatitis in the chronic abdominal pain syndrome group and the initial finding minimal change chronic pancreatitis group.

Clinical Variables	CAPS N=38			Initial findings MCEUS N= 28			Total N=66		
	No AP n=25	Prev AP n=13	P value	No AP n=7	Prev AP n=21	P value	No AP n=32	Prev AP n=34	P value
Gender: Men	10 (40.0%)	9 (69.2%)	0.087	4 (57.1%)	13 (61.6%)	>0.999	14 (43.8%)	22 (64.7%)	0.087
Age at first symptoms Median (IQR) years	41 (30-46)	45.5 (36.8-48.3)	0.249	31 (22.5-36)	39 (30.3-45)	0.114	38.5 (28-45.3)	41 (34.3 - 46.5)	0.304
Duration of pain Median (IQR) years	10 (6-14)	10 (4-15)	0.711	13 (9.5-15)	6 (3-9)	**0.026**	10 (4-15)	7 (4.5-11.5)	0.259
Clinical exocrine insufficiency	3 (12.0%)	3 (23.1%)	0.392	0 (0%)	5 (23.8%)	0.290	3 (9.4%)	8 (23.5%)	0.123
Pancreatic enzyme supplements	18 (72.0%)	7 (53.8%)	0.301	6 (85.7%)	9 (42.9%)	0.084	24 (75.0%)	16 (47.1%)	**0.020**
Insulin dependent diabetes mellitus	0 (0%)	3 (23.1%)	**0.034**	0 (0%)	2 (9.5%)	>0.999	0 (0%)	5 (14.7%)	0.054
Oral hypoglycaemics for diabetes mellitus	3 (12.0%)	1 (7.7%)	>0.999	0 (0%)	0 (0%)	N/A	3 (9.4%)	1 (2.9%)	0.348

Consumed ≥62 units per week of alcohol for ≥1 year	11 (44.0%)	2 (15.4%)	0.148	1 (14.3%)	6 (28.6%)	0.639	12 (37.5%)	8 (23.5%)	0.217
Alcohol units consumption per week in excess drinkers Median (IQR)	160 (100-240)	221 (143.3-299.8)	>0.999	90 (90-90)	116 (80.5-450)	0.611	130 (97.5-220)	116 (70-423.5)	0.816
Ever smoker	16 (64.0%)	9 (69.2%)	>0.999	5 (71.4%)	13 (61.9%)	>0.999	21 (65.6%)	22 (64.7%)	0.938
Current smoker	8 (32.0%)	4 (30.8%)	>0.999	3 (42.9%)	3 (14.3%)	0.144	11 (34.4%)	7 (20.6%)	0.209
Number of pack years in ever smokers Median (IQR)	18 (13.8-30)	25 (12.5-30)	0.705	10 (7.5-18)	11 (6.5-17)	0.884	16 (10-28)	13.5 (8.5-20)	0.364
Gainful employment	5 (20.0%)	1 (7.7%)	0.643	2 (28.6%)	8 (38.1%)	>0.999	7 (21.9%)	9 (26.5%)	0.663
Regular strong opiates	19 (76.0%)	12 (92.3%)	0.385	6 (85.7%)	8 (38.1%)	0.077	25 (78.1%)	20 (58.8%)	0.092
Daily strong opiates	13 (52.0%)	8 (61.5%)	0.575	3 (42.9%)	5 (23.8%)	0.371	16 (50.0%)	13 (38.2%)	0.336
Cholecystectomy	5 (20.0%)	5 (38.5%)	0.263	3 (42.9%)	5 (23.8%)	0.371	8 (25.0%)	10 (29.4%)	0.688
Deaths during study period	1 (4.0%)	1 (7.7%)	>0.999	0 (0%)	0 (0%)	N/A	1 (3.1%)	1 (2.9%)	>0.999

Table 8. EUS features in patients with a history of acute pancreatitis in the chronic abdominal pain syndrome group and the initial finding minimal change chronic pancreatitis group.

EUS Standard Criteria (ref)	Acute Pancreatitis in Chronic Abdominal Pain Syndrome	Acute Pancreatitis in Initial finding of MCEUS	*P value	Total
Patients with history of acute pancreatitis	13 (34.2%) of 38	21 (75.0%) of 28	**0.001	34 (51.5%) of 66
Patients with AP who had EUS	9 (69.2%)	21 (100%)		30 (88.2%)
EUS reports diagnosing ifMCCP without listing specific EUS criteria	0 (0%)	7 (33.3%)		7 (23.3%)
EUS reports listing specific features including negative features	9 (100%)	14 (66.7%)		23 (76.6%)
PARENCHYMAL FEATURES				
Hyperechoic foci	1 (11.1%)	8 (57.1%)	**0.040**	8 (34.8%)
Hyperechoic strands	1 (11.1%)	5 (35.7%)	0.340	6 (26.1%)
Lobularity Without honey combing With honey combing	 3 (33.3%) 0 (0%)	 6 (42.9%) 3 (21.4%)	 >0.999 0.253	 9 (39.1%) 3 (13.0%)
Pseudocysts	2 (22.2%)	3 (21.4%)	>0.999	5 (21.7%)
DUCTAL FEATURES				
Main duct dilatation	0 (0%)	0 (0%)	-	0 (0%)
Duct irregularity	0 (0%)	2 (14.3%)	0.502	2 (8.7%)
Hyperechoic margins	0 (0%)	6 (42.9%)	**0.048**	6 (26.1%)
Visible side branches	0 (0%)	0 (0%)	-	0 (0%)
Intraductal stones	0 (0%)	0 (0%)	-	0 (0%)
Number of patients any EUS feature	5 (55.6%)	14 (100%)	**0.014**	16 (82.6%)

**P value with Bonferroni correction is significant, p<0.0029.

NB some patients will have had more than one EUS feature.

3.4.3 Patients demonstrating disease progression. Comparison of MCEUS patients progressing to definite chronic pancreatitis with non-progressors (Stage D).

In addition to the 28 patients discussed in the above sections who had some MCEUS features but did not progress to definitive CP, a further 12 patients were identified who were initially found to have MCEUS changes but went on to demonstrate clear progression to radiologically or histologically proven CP. These 12 patients were classified within the 689 patients with proven CP.

The demographics and clinical features of these 12 (9 male: 3 female) MCEUS patients who progressed to CP are presented in table 9.

Table 9. Demographics of patients with MCEUS findings who went on to progress to established CP.

Age at first symptoms, years ,gender	Aetiology	No. of CP risk factors	Alcohol excess	Smoking status	Main symptoms	Diabetic status	Exocrine insufficiency	Daily opiates	Employed	Diagnostic criteria			Diagnostic modality	*Time to diagnosis from MCEUS (months)	**No. of imaging tests to diagnosis
										Standard	Rosemont	Japanese System			
31 Male	Alcohol	2	Yes	Current	Pain, anorexia	None	Yes	Yes - Weak	No	3 criteria = Indeterminate	3 minor criteria = Normal	2 EUS criteria 4 Clinical criteria = Possible CP	CT	30	4
39 Female	Hyper-calcaemia	2	No	Current	Pain	None	Yes	Yes - Strong	No	4 criteria = Indeterminate	Major B criteria 3 minor criteria = Suggestive	3 EUS criteria 3 Clinical criteria = Early CP	CT	25	3
40 Male	Alcohol	2	Yes	Current	Pain	None	Unknown	Yes - Strong	No	2 criteria = Normal	2 minor criteria = Normal	2 EUS criteria 2 Clinical criteria =Normal	CT	76	2
35 Female	Alcohol	3	Yes	Ever	Pain, anorexia	None	Yes	Yes - Strong	No	3 criteria = Indeterminate	3 minor criteria = Normal	1 EUS criteria 4 Clinical criteria = Possible	CT	35	1
22 Male	Alcohol	3	Yes	Current	Pain, anorexia	Insulin	Yes	Yes - Strong	No	4 criteria = Indeterminate	1 major B 3 minor criteria = Indeterminate	3 EUS criteria 4 Clinical criteria = Early CP	CT	24	5
25 Male	Idiopathic no mutation	1	No	Never	Pain, steatorrhoea	Insulin	Yes	Yes - Strong	No	4 criteria	3 minor criteria = Normal	3 EUS criteria	CT	28	3

										= Indeterminate		3 Clinical criteria =Early CP			
42 Male	Alcohol	3	Yes	Current	Pain	None	No	Yes - Weak	No	N/A	N/A	N/A EUS criteria 3 Clinical criteria = Possible CP	CT	48	4
66 Male	Idiopathic (no mutation)	0	No	Never	Pain	None	Yes	Yes - Weak	Retired	2 criteria = Normal	2 minor criteria = Normal	2 EUS criteria 0 Clinical criteria = Normal	MRCP	7	1
50 Male	Alcohol	2	Yes	Current	Pain	Oral drugs	Yes	Yes - strong	No	3 criteria = Indeterminate	4 minor criteria = Indeterminate	1 EUS criteria 3 Clinical criteria = Possible CP	CT	34	3
Unknown Female	Alcohol	2	Yes	Current	Weight loss, Pain	None	No	Unknown	Retired	N/A	N/A	N/A EUS criteria 2 Clinical criteria	CT	49	2
34 Male	Idiopathic (no mutation)	2	No	Current	Pain	None	No	Yes - Weak	Yes	3 criteria = Indeterminate	3 minor criteria = Normal	2 EUS criteria 2 Clinical criteria = Normal	CT	40	1

53 Male	Alcohol	3	Yes	Current	Pain, anorexia	None	Yes	Yes - Weak	No	N/A	N/A	N/A EUS criteria 4 Clinical criteria = Possible CP	CT	19	4

N/A= not available; *median (IQR) time to progression= 32 (24.5-42) months; **median (IQR) number of if investigations = 3 (IQR 1.75-4)

The comparison of clinical features in MCEUS patients with and without progression to definite CP is summarised in table 10. The median (IQR) age at first symptom in non-progressive cohort was 39 (32.5-46) years, eight (67%) patients had a history of confirmed alcohol excess (> 62 units / week for >1 year), 10 (83%) had a positive history for tobacco smoking, and seven (58%) had a history of AP attack/s. Based on full clinical assessment and consenting, seven patients underwent genetic testing with no mutations identified. In confirmed disease progression, the median (IQR) follow-up period from MCEUS to definitive CP was 30 (18.75-36.5) months.

The non-progressive group (n=28, 17 males; p=0.488), the median (IQR) age at first symptoms was 38 (25-43) years (p=0.488). Seven (25%) had a history of confirmed alcohol excess (> 62 units / week for >1 year) (p=0.031), 18 (64%) were ever smokers (p=0.446) and six (21%) were current smokers (p=0.004). Twenty-one (75%) had a history of AP attack/s (p=0.453), 20 (71%) underwent genetic testing with four identified mutations (p>0.999); two heterozygous SPINK-1 N34S and two CFTR ΔF508. The median (IQR) clinical follow-up period without radiological progression was 35 (7-61.5) months.

3.7.1.4 Regression of MCEUS changes

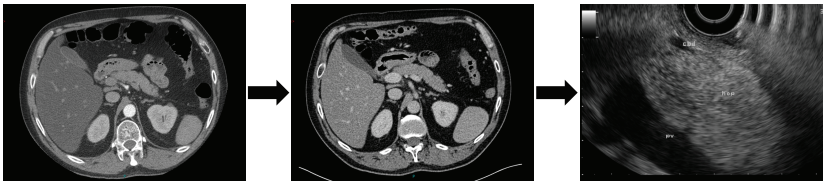
Five patients demonstrated complete resolution of MCEUS related changes during clinical follow up. These patients underwent repeat EUS investigation at a median (IQR) of 3.0 (2.1-6.0) years between tests. These five patients were therefore included within the ‘non-progressive’ group.

Table 10. Comparison of patients with MCEUS who progressed to definite chronic pancreatitis with those who did not progress.

Clinical Variables	MCEUS progressing to definite CP N=12	MCEUS without progression N=28	*P value
Gender (Male: Female)	9:3	17:11	0.488
Age at first symptoms Median (IQR) years	39 (32.5-46.0)	38 (24.25-44.25)	0.488
Consumed ≥62 units per week of alcohol for ≥1 year	8 (67%)	7 (25.0%)	0.031
Ever smoker	10 (83%)	18 (64%)	0.446
Current smoker (%)	9 (75%)	6 (21%)	0.004
One attack of acute pancreatitis	7 (58%)	21 (75.0%)	0.453
Pancreatic Exocrine Insufficiency	8 (67%)	5 (18%)	0.002
Pancreatic Surgery	6 (50%)	0 (0%)	<0.001
Deaths during study period	3 (25%)	0 (0%)	0.022

From the group demonstrating disease progression on follow up; Eight (67%) patients demonstrated symptoms of pancreatic exocrine insufficiency (PEI), compared with five (18%) without progression (p=0.002). Six (50%) patients with progression required pancreatic surgery compared with none (0%) without progression (p<0.001). Examples of the imaging seen in re-classified CAPS patients, regression of MCEUS changes a, and progressive disease are given in Figure 8. Three (25%) patients with progression died during follow-up all of causes related to chronic pancreatitis or CP complications, no patients died in the non-progression group (p=0.022).

Figure 2 (A). Imaging from a patient previously diagnosed with chronic pancreatitis, now reclassified as chronic abdominal pain syndrome

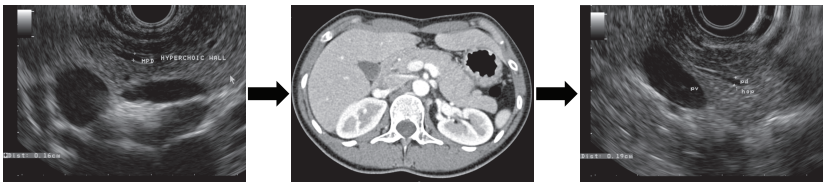


CT 2010: Normal pancreas, forshortened tail

CT 2012: Stable appearance of pancreas

EUS 2014: Normal EUS

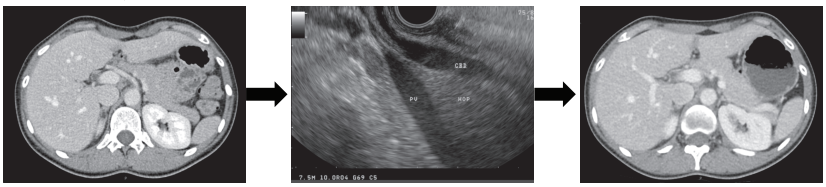
(B). Regression of initial finding of minimal change of chronic pancreatitis on EUS examination



EUS 2009: MCEUS; hyperechoic MPD margin with hyperechoic areas

CT 2011: Normal pancreas

EUS 2014: No criteria for CP

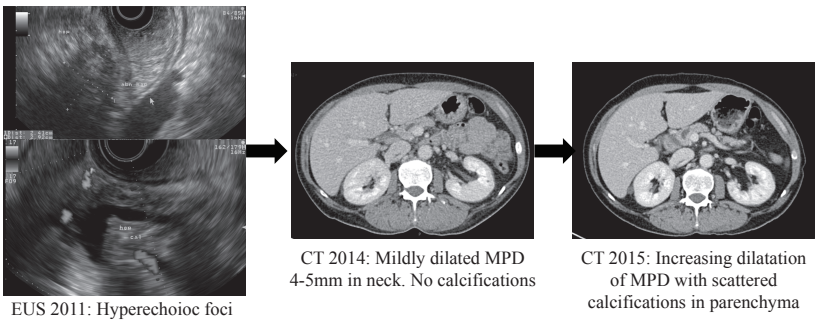


CT 2014: Normal pancreas

EUS 2015: Normal EUS

CT 2016: Normal pancreas

(C). Example of a patient with disease progression to chronic pancreatitis



EUS 2011: Hyperechoioc foci with shadowing and lobularity. Heterogeneous HOP

CT 2014: Mildly dilated MPD 4-5mm in neck. No calcifications

CT 2015: Increasing dilatation of MPD with scattered calcifications in parenchyma

Figure 8. Comparable Imaging examples from CAPS, regression, and progression patients.

A) An example of imaging in a patient reclassified as CAPS. B) and C) demonstrate good examples of the radiological imaging from this subgroup of patients with regression and progression of MCEUS features (8B and C respectively)

3.8 Discussion

Refining the diagnosis of CP within the Liverpool CP database cohort within the context of the mechanistic definition alongside the 5-stage conceptual model of CP disease progression, affords further insight as to why certain patients may have been prematurely or incorrectly diagnosed with CP. The chronic pancreatitis patient cohort seen in the tertiary referral centre at the Royal Liverpool University Hospital represents a cohort of patients presenting late in the CP disease progression pathway, typically with 'established' or 'end stage' disease. The vast majority (n=689, 85%) of the Liverpool cohort would already be classified as Stage D/E on initial presentation to the Liverpool pancreas services. The exact reasons behind this are unclear, however they are likely to be multifactorial, complex and associated with the region's high levels of social deprivation, substance misuse and poor health care access. Liverpool's role as a supra-tertiary referral centre for pancreatic diseases and pancreatic cancer along with its international reputation for pancreatic research and specialist interest in inherited diseases of the pancreas would also influence the type of referrals the centre receives. The subgroup of interest in terms of disease progression, are those that were prematurely, incorrectly or misdiagnosed.

3.8.1 Risk factors

All the patients in both the CAPS and the MCEUS group had a similar number and distribution of risk factors. Thirty-two of the 38 patients with CAPS and 26 of the 28 patients with MCEUS features of CP had between one and three risk factors associated with chronic pancreatitis. The mechanistic definition of chronic pancreatitis discusses the 'essence' of CP as "a pathologic fibro-inflammatory syndrome of the pancreas in individuals with genetic, environmental and/or other risk factors who develop persistent pathologic responses to parenchymal injury or stress". Risks factors form a central part of this concept and the acknowledgement of the presence or exposure to risk factors for chronic pancreatitis provides insight into the most likely underlying

aetiology of chronic pancreatitis with implications for potential future therapeutic targets (10).

With further follow-up and a median of four investigations per patient, no patient from either the CAPS or the non-progression MCEUS group displayed any imaging evidence of definite chronic pancreatitis and in 5 cases EUS imaging reverted to normal. These data support the view that the presence or absence of one or more risk factors alone cannot be used to infer a diagnosis of early chronic pancreatitis in the absence of characteristic imaging findings or histopathology. This supports the findings of Konings et al. who assessed the presence of MCEUS features of CP in a cohort of patients undergoing EUS surveillance at risk of pancreatic ductal adenocarcinoma. Despite a high prevalence of MCEUS features on initial imaging (86%) they found no significant association between the presence of risk factors for CP and the presence of >4 MCEUS features on EUS surveillance (141). The recent publication of international consensus statements for early CP also recognise this evolving view stating that although genetic variants and/or environmental risk factors such as AP are important/significant RF for chronic pancreatitis, their presence alone is neither necessary nor sufficient to make a diagnosis (142).

3.8.2 Acute pancreatitis and timing of investigations

Features of post-acute pancreatitis seen on co-axial imaging and EUS can take 12 months or more to resolve (in the absence of necrotising pancreatitis at the initial attack), usually lagging behind the resolution of clinical symptoms. In the present series, of the 52 patients reclassified with another diagnosis, 23 had resolving post- acute pancreatitis imaging features and 12 had recurrent acute pancreatitis with resolution of symptoms and imaging findings between attacks, none of these patients' developed features of chronic pancreatitis during the study follow-up period.

Amongst the 66 patients with CAPS and non-progression of MCEUS features misdiagnosed with chronic pancreatitis there were 34 patients with at least a

single attack of acute pancreatitis. Patients in the MCEUS group were significantly more likely to have had a previous attack of acute pancreatitis compared with those reclassified as chronic abdominal pain syndrome. In addition, patients with a previous attack of acute pancreatitis who went on to undergo EUS were significantly more likely to have findings of MCEUS if the episode of pancreatitis was within 12 months of the EUS ($p = 0.007$). These findings may suggest that patients with previous acute pancreatitis are more likely to be diagnosed with MCEUS features of early chronic pancreatitis and that the risk is highest for patients undergoing EUS within one year of acute pancreatitis. Care must therefore be taken to avoid over-interpreting EUS features particularly in patients with a recent attack of acute pancreatitis.

3.8.3 MCEUS progression and ongoing risk factor exposure

From review of the patients included in the Liverpool CP database, 40 with MCEUS features and abdominal pain were identified, and of these 12 progressed to definite CP after a median radiological follow-up approaching three years. In five cases the minimal change EUS findings resolved on follow up. As far as we are aware this is the first description of resolution of MCEUS changes published in the literature (143). Patients from the Liverpool cohort who demonstrated disease progression were significantly more likely; to have excess alcohol consumption (> 62 units / week), to be current smokers, to develop symptoms of pancreatic exocrine insufficiency (measured as patient reported steatorrhoea), require pancreatic surgery and had a higher mortality than those who did not progress.

A similar relationship between continued exposure to toxic risk factors and disease progression was seen in a more recent published multicentre, prospective Japanese study has also shown that progression from early chronic pancreatitis to established chronic pancreatitis was very rare and only seen in 4 out of 83 (4.8%) patients with ‘early CP’ who were followed up for 2 years (144). Of the 4 patients in the Japanese study who demonstrated disease progression; all were male, all had a history of alcohol excess with

continued alcohol consumption, and all were ‘ever’ smokers (3 current, 1 ex-smoker) (144).

These data supported by further prospective follow up data from Japan suggests that risk factor modification with smoking cessation and abstinence from alcohol in the cohort of patients with MCEUS features of early chronic pancreatitis may prevent disease progression towards established chronic pancreatitis.

3.8.4 The role of EUS in the diagnosis of early CP

Despite EUS being a sensitive imaging modality able to detect subtle pancreatic ductal and parenchymal changes which may be the earliest visible features of CP, there are several limitations which hamper its relevance and diagnostic specificity. A number of the EUS abnormalities associated with the appearance of ‘early CP’ have now been shown to also be found in patients who do not have CP. These changes can be seen in increasing age, smoking, and diabetes pancreatopathy and could result in false positive results. In addition to the challenges of intra and interobserver variability, interpreter bias is heavily influenced by pre-test probability of a disease. EUS is heavily operator dependant and the individual performing the examination will be aware of the patient’s history, risk factors and overall likelihood of having CP. The investigation request may also state the likely/assumed diagnosis. Interpreter bias may therefore influence the interpretation of the EUS results.

Due to these limitations, EUS cannot be seen as an imaging surrogate to histology. Furthermore, a recent international consensus statement by expert histopathologists confirmed that histology is not the gold standard for diagnosis CP.

3.9 Conclusion

Chronic pancreatitis is a very heterogenous disease. Although many patients with CP within the Liverpool CP cohort have advanced or end staged CP at the time of presentation to the clinical services, a small group of patients were

identified with ‘early CP’ or MCEUS like changes. Consistent with data from a prospective but smaller Japanese study only a small number of patients progressed, the majority stayed static and some ‘early’ cases regressed.

Risk factors play a key role in the diagnosis of CP and are especially relevant in the mechanistic definition of CP however, when the presence of a risk factor is taken in isolation, it is neither sufficient nor mandatory to make a diagnosis of CP.

The role of EUS in the diagnosis of early CP within the scope of the mechanistic approach is that of a biomarker of disease progression opposed to an imaging modality which defines the character of a disease by fibrosis. Care must be taken in the interpretation of EUS findings within the 12 months following an AP attack.

Chapter 4: Candidate biomarkers in CP - a collaborative project with Aalborg university

4.1 Overview

Pancreatic function testing and cross-sectional imaging are currently used to guide clinicians when diagnosing chronic pancreatitis. They also form a significant part of some published CP diagnostic criteria (9, 96, 145). These methods are expensive, time consuming and can be invasive for the patient. Current techniques also lack diagnostic accuracy. There is a need for cheaper, less invasive, and more accurate alternative diagnostic adjuncts. Pancreas specific amylase (Pancreas-α-amylase (Amy-P)) can be routinely measured in serum, plasma, and urine. This study investigated the utility of pancreas-specific plasma amylase for assessment and diagnosis of chronic pancreatitis.

Through networking at international society meetings and presenting work at conferences, academic relationships were fostered with the pancreatic research team at Aalborg University.

As part of this thesis blood/serum samples collected from well characterised patients consented and recruited to the Liverpool Chronic pancreatitis database and biobank were identified, prepared, and sent along with anonymised relevant clinical information to Aalborg University to be included in a collaborative project combining samples from Denmark and the UK. Patient blood samples collected at Aalborg University hospital were used to form the discovery cohort and the samples provided by Liverpool formed the validation cohort. All laboratory biochemical analyses were performed at Aalborg University Hospital.

Discovery cohort: The blood samples used to form the discovery cohort were collected at Aalborg University Hospital, Denmark. Patients referred with a clinical suspicion of CP between 2013-2017. Patients with a diagnosis of

definitive or probable CP according to the M-ANNHEIM classification system were eligible for inclusion to ensure the full spectrum of disease was covered.

Validation cohort: We provided 70 patient blood samples which formed the validation cohort from well characterised patients from within the Liverpool CP biobank collected between 2011-2017.

Reference population: 94 healthy donors from Aalborg.

This work has been published by Oleson et al. This manuscript contains the materials, methods, and results of the study (146).

4.2 Discussion

This collaboration investigated the diagnostic performance of pancreas-specific plasma amylase level in the assessment of CP.

A modified version of the M-ANNHEIM clinical staging score was used to further classify the study's CP population (table 11).

Table 11. The modified M-ANNHEIM clinical staging score

Modified M-ANNHEIM Classification (147)	
Disease stage	Clinical features
Stage I	No evidence of EPI or DM
Stage II	Evidence of EPI or DM
Stage III	Evidence of EPI and DM Painful complete pancreatic insufficiency
Stage IV	Evidence of EPI and DM Non-painful complete pancreatic insufficiency

N.B Stage III & IV were grouped for statistical analysis.

This study identified significant and independent associations at multivariate analysis between low plasma amylase levels and the presence of diabetes mellitus and exocrine pancreatic insufficiency. Furthermore, this association did not appear to be directly related to CP disease duration.

Pancreas specific plasma amylase was highly sensitive (94%) and moderately specific (59%) for diagnosing chronic pancreatitis, with an optimal plasma level defined at 17.3 U/l. Further stratified analysis based on a modified M-ANNHEIM clinical stage demonstrated the increased diagnostic performance of plasma amylase in advanced chronic pancreatitis. The main study findings were consistently replicated within the validation cohort comprising of the patient samples provided by Liverpool.

The high sensitivity and moderate specificity of plasma amylase means that currently, a low plasma amylase level could be used to ‘rule in’ a diagnosis of CP. Additionally, a normal plasma amylase level would not exclude a CP diagnosis. One key finding of this study was the improved diagnostic accuracy with increasing disease severity. This study used a Modified M-ANNHEIM disease classification system which is largely based on pancreatic function and the presence of symptoms, whereas other systems use pancreatic morphology defined on imaging to establish disease severity. There is no clear relationship between disease morphology on imaging and symptomatology in CP therefore, for the purposes of this study, we felt the use of a disease severity classification which was based more heavily on current clinical investigations would provide more transferable/ relatable / real world results.

The future clinical implications of this work are promising but currently unclear. The diagnostic utility of plasma amylase in CP needs to be further validated in large independent studies, and these results need to be replicated in low CP prevalence populations such as those seen outside of specialist pancreatic tertiary referral centres (e.g., primary and secondary health care settings).

Low plasma pancreas specific amylase could be included in future diagnostic criteria for CP with the caveat that it offers a specific but insensitive biomarker of CP. Patients found to have a low plasma amylase could also be targeted for a focused assessment for previously undiagnosed malnutrition and diabetes.

Chapter 5: The International CP guidelines

Consensus voting outcomes and results from the 'working groups' comprising the international consensus guidelines for chronic pancreatitis (ICGCP) have been published in several peer reviewed manuscripts which are listed in Appendix 2:

5.1 Introduction

Independently produced guidelines for CP have been previously published. However, the concept of a truly international document is entirely novel and the need for such a piece of work is clear. Data presented and discussed in chapter 3 highlighted the challenges of establishing a diagnosis of CP especially in early disease stages and differentiating CP from alternate diagnoses that may share similar risk factors and symptom profiles. The recent flurry of research interest in CP has led to many developments in our understanding of the disease processes however, until the International pancreatic community can form consensus opinions of the key topics in CP, then data sharing, collaboration between nationalities and CP research in general will not be able to progress.

5.1.1 Previous guidelines

There are already guidelines for CP in existence (9, 145, 148-150). Some focus purely on specific elements of chronic pancreatitis, for example the American Pancreatic Association (APA) evidence-based report on diagnostic guidelines in CP (9) deals primarily with the diagnosis. Other guidelines have been endorsed by individual societies such as the HanPanEU European consensus (148), while some have been produced on a national level including the German S3 guidelines (150) and the Spanish Pancreatic Club recommendations for the diagnosis and treatment of CP (151, 152). While one cannot fail to be impressed by the thoughtful, thorough, and conscientious

approach taken by the authors of these previous CP guidelines, the process for panel and topic selection was not always entirely transparent and tended to focus on local or national expertise and experience. The complexity of CP and the differences in disease manifestations between countries and populations requires the perspectives of international experience and expertise. This is particularly relevant to the challenges facing the international community addressing the definition and diagnosis of early CP where clinicians, scientists and researchers globally need to consider new ways to frame the disease.

5.1.2 A new approach

Previous attempts to gain a consensus view on the hot topics of CP have stalled and a fresh approach was needed. The arduous but necessary process of challenging established misconceptions began with the excellent work by the APA guideline group (9). This body of work provided a sound foundation for the international guidelines to build on. International consensus guidelines for chronic pancreatitis (ICGCP) aim to be different from previous guidelines by bringing recognised international experts in pancreatology together to produce a document that would be clinically focused. The group felt there was a need for a more pragmatic basis for patient diagnosis and management but also a need to facilitate the assessment of patients at the earliest opportunity in the CP disease process to gather information crucial to the development of newer pharmacological and other therapies. As a start David Whitcomb (with Luca Frulloni, Pramod Garg, Julia B. Greer, Alexander Schneider, Dhiraj Yadav, Tooru Shimosegawa) led a new initiative “Chronic Pancreatitis: An international draft consensus proposal for a new mechanistic definition” (10). This new mechanistic definition heralded a paradigm shift in the approach to the ‘CP problem’ and served as inspiration for the ICGCP core committee.

The definition was proposed and hotly debated at several international meetings and forums and following voting at several of these meetings, and an overall Delphi consensus process, this definition was adopted and endorsed by the international pancreatic community.

The International process outlined in this chapter follows on from the international adoption of the novel mechanistic definition of chronic pancreatitis and conceptual model of disease initiation and progression proposed by Whitcomb et al. This project aimed to create a fresh clinical and pragmatic approach towards the diagnosis of CP at all disease stages, and disease management with focus on the most important complications of CP. This process will assist a more pragmatic basis for patient assessment and treatment, but also to help accelerate the assessment and hence the development of novel therapies and treatment modalities.

5.2 Scope of the guidelines

The initial proposed scope of the International CP Guidelines is summarised below. It was decided at the earliest point in the processes concept that these guidelines would not seek to cover any aspect of Autoimmune Pancreatitis.

The aim of the International Guidelines on Chronic Pancreatitis was to create a consensus guideline that is truly international and multidisciplinary, covering from development and early diagnosis to progression and treatment of CP.

- Disease mechanism based
- Truly international and globally applicable
- Clinically pragmatic
- Multidisciplinary

Working group topics:

- The definition of Chronic pancreatitis (covered by the EPC milestone meeting)
- The definition of Early CP
- Risk factors for CP
- Symptoms of CP
- Diagnostic Cross-Sectional Imaging and Severity Scoring of Chronic Pancreatitis

- The role of diagnostic endoscopic ultrasound in the management of chronic pancreatitis
- Pain management in CP
- Medical management of CP
- Indications and timings of surgical interventions in CP
- Role of Interventional endoscopy in CP
- The role of Total pancreatectomy with auto-transplantation in CP
- Surveillance for pancreatic cancer in chronic pancreatitis
- histopathology of chronic pancreatitis

5.3 Methods

5.3.1 My role in the guideline process

My role within this process started at the point of its conception. I was the guideline coordinator, I was placed on the core committee, the main working group, and I was also a panel member for several of the working groups. My responsibilities included; designing a pragmatic 'modified Delphi' process that would complement the guideline process and how we anticipated it would work, topic selection, deciding on the method for grading evidence that would be set throughout the process, working with the department of statistics at the Liverpool Cancer Trials Unit to design and commission the dedicated online voting platform for the modified Delphi process, identifying, approaching and inviting key international personalities and experts to participate and chair working groups, guiding the working groups though the various stages of the Delphi process, Data collection, Data analysis, distributing results to core committee and working parties, and manuscript drafting and editing.

5.3.2 Core working group and panel selection

In 2016, the Presidents of the International Association of Pancreatology (Tooru Shimosegawa), American Pancreatic Association (Carlos Fernandez-Del Castillo), Japan Pancreas Society (Shuiji Isaji) and the European Pancreatic Club (John P Neoptolemos) under the chairmanship of David C

Whitcomb collaborated to develop the first truly International Consensus Guidelines for CP. Following an initial scoping call, there was excellent support for the process from the four major international pancreatic society's presidents (EPC, IAP, JPS and APA) and members alike and the process received official society endorsement. From the positive responses and nominations, individuals with the expertise and international reputation deemed suitable to potentially chair a group were invited to take on this role. Further nominations and volunteers were accepted thought the process until the consensus statement preparations had been completed. The international experts who contributed to the ICGCP process and their roles are listed in Appendix 1.

5.3.3 Topic selection

Producing guidelines on CP is unquestionably a considerable task. The core committee for the working group therefore decided to divide the work into more manageable sections. Each section focused on the key topics of CP, which were felt would benefit from consensus statements. The core committee identified international experts to ensure multidisciplinary representation from most regions of the world, and they were invited to contribute work to their respective areas. Calls for volunteers to participate in the process were also circulated around the four International Societies.

5.3.4 The Delphi process

The Delphi technique is a structured group process which is an accepted, reliable method of exploring expert opinion and experiences to establish a scientific basis for consensus on a topic. The method was initially developed to predict cold war enemy attack probabilities but is now an established way to determine consensus in a defined clinical area.

The Delphi process involves systematic canvassing of expert opinion over a pre-defined number of rounds using open and closed ended questions. Individual expert responses are collated and reported as a statistical response, these results are then fed back to the panel. Participants can adjust their initial

ratings based on this feedback in a number of subsequent iterations (153). These rounds are continued until a priori defined criterion is met (i.e consensus is achieved or agreed number of rounds completed) (154).

The Delphi investigators should identify the scope of the issues discussed as part of the process. This can result in bias and any consensus reached could be distorted (155). A further criticism is that the panel members do not meet in person and thus discussion cannot take place (155). This process employed the modified Delphi process with scheduled key milestone meetings allowing the working party to meet face to face, feedback on topics included and discuss any challenges or contentious fields with the core committee. Of note, no voting took place during these CP sessions to ensure anonymity during the voting process is maintained.

Following a trial Delphi round on tester questions the core committee agreed that the ICGCP modified Delphi process would close following the completion of 2 rounds of voting, or on reaching consensus. The possibility of further rounds of voting where explored, however areas with 'weak' levels of agreement were found to be very challenging fields often with polarising views and minimal progress or compromise was achieved after meetings. It was agreed that further discussion, editing of statements or voting would not change the result.

5.3.5 Statistics and definition of consensus

For the purposes of the ICGCP process, consensus was defined as 'consistency of opinion amongst the panel of experts. Cronbach's alpha is the statistical index of choice to quantitatively evaluate the reliability of a summation of entities (in this case a collection of expert opinions) (156). A high correlation between expert responses demonstrates internal consistency (155). Cronbach's alpha can be directly interpreted as the correlation expected between the present panel and a second panel selected from the same population of content experts.

A Cronbach's alpha >0.90 has been suggested as necessary for clinical diagnostic scales (156, 157). Previous studies have employed a Cronbach's a of _0.80 as representative of an acceptable measure of internal reliability (158-160).Best practice guidelines suggest that criteria for consensus should be defined in advance (161, 162) Therefore, consensus was predefined as Cronbach's a _0.80.

5.3.6 Process outline

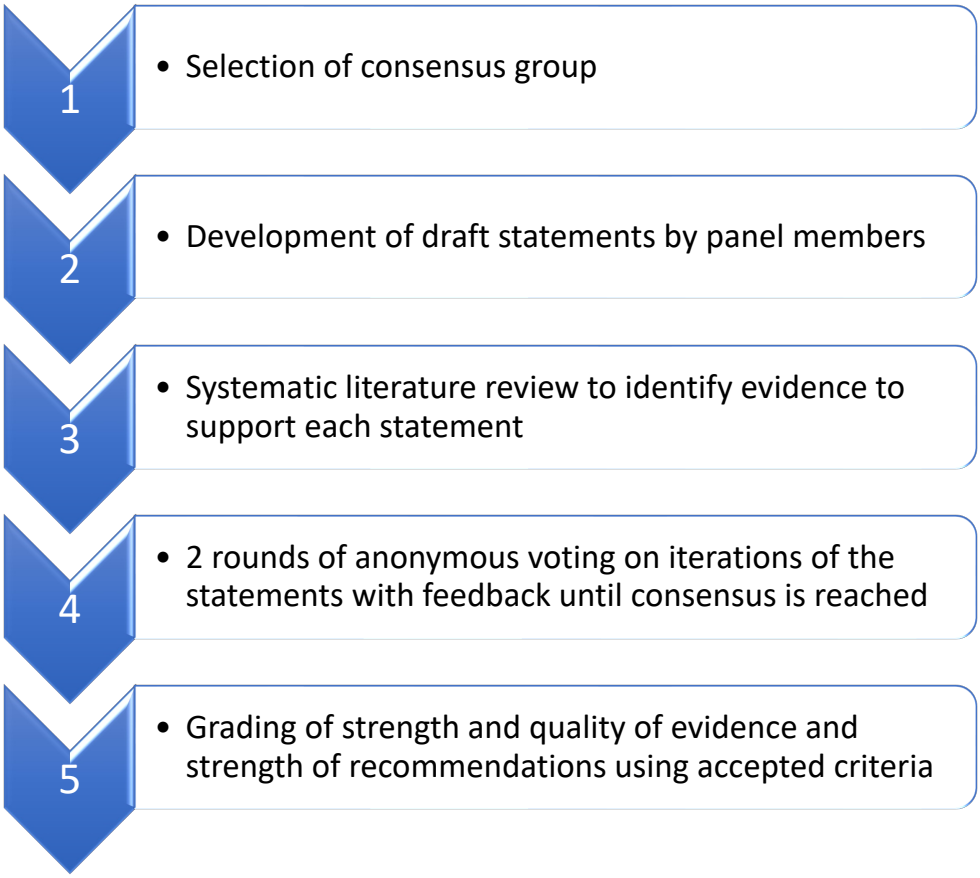


Figure 9. The step process for the development of the ICGCP.

5.3.7 Rating the quality of evidence available

During the conception of the International CP guidelines, the chairs and guideline co-ordinator approached the core committee to vote on their preferred system for rating the quality of the best available evidence and developing health care recommendations, which would be used as the basis for the International CP guideline recommendations. Voting was between an approach proposed by the Grading of Recommendations, Assessment, Development and Evaluation (GRADE) Working Group, the OXFORD rating, and the SIGN (Scottish Intercollegiate Guideline Network). The consensus was in favour of adopting a GRADE approach, as adapted for "UpToDate".

(http://www.uptodate.com/home/grading- tutorial). for topics lending themselves to evidence-based statements.

The core committee provided a structured format for performing systematic reviews. This included utilising the bibliographic databases MEDLINE, EMBASE and the Cochrane Central Register of Controlled Trials, to identify relevant papers for inclusion. It was strongly recommended that each working group used the 'GRADEpro' online application tool to assist in achieving consistent evaluation of the level of evidence and clinical implications according to the GRADE guidelines.

The quality of evidence supporting the guideline statements was graded as (i) "high" if there was very low probability of further research completely changing the presented conclusions, (ii) "moderate" if further research may completely change the conclusions, (iii) "low" if further research is likely to change the presented conclusions completely. The term "very low" (iv) could be used if new research will most probably change the presented conclusions completely; however, the term was not used in the present work.

The strength of the recommendation was classed as "weak/ conditional", "strong" or "not applicable". This considered the quality of evidence, the translation of evidence into clinical practice, and any relevant uncertainties relating to population risk.

The guideline development process evolved over several milestone meetings at subsequent society conferences hosted throughout 2016.

5.3.8 Milestone meetings

To assist co-ordinating concurrent activity relating to the ICGCP development process, the European Pancreatic Club 48th annual meeting July 6-9th 2016, Liverpool, UK was highlighted as a key development milestone meeting for the entire ICGCP process. Good progress was made, and a further series of initial meetings continued during the 20th Scientific Meeting of the International Association of Pancreatology in conjunction with the 47th annual meeting of the Japan Pancreas Society and the 6th meeting of the Asian Oceanic Pancreatic association August 4-8th 2016, Sendai International Center, Japan; American Pancreatic Association 47th annual meeting October 26-9th, Boston MA, USA; Pancreas Fest July 31- August 1, 2017; 21st International Association of Pancreatology in conjunction with the Latin American Pancreatic Study Group first joint meeting September 28th-30th, 2017 in Buenos Aires, Argentina.

A variety of guideline meetings were hosted during these milestone events. DCW was invited to present the progress on the main program at all the key meetings. In addition to this there were several breakout sessions and face to face meeting to allow participants to raise any questions, concerns or challenges they were facing. Groups also met to finalise statements and hold discussions in person.

5.3.9 Online voting platform

Voting was completed via a unique dedicated online voting portal created with support from Information Technology services and the Liverpool Cancer Trials Unit (LCTU). Voting was essentially anonymous, with only the guideline co-ordinator having access to individual results (to ensure all participants had completed voting and any duplications etc could be easily resolved).

5.3.10 Voting on level of agreement

To formalise the level of objective support from the participating international expert panel, members of the working groups were asked to complete online voting using a nine-point Likert scale from 1, “strongly disagree”, to 9, “strongly agree” on their level of agreement with the recommendations and the provided GRADE score. Voting results were used to calculate Cronbach's alpha reliability coefficient and were classified under “agreement” as either; strong (80% of votes were 7 or above), conditional (65% of votes were 7 or above), and weak (<65% of votes were 7 or above).

When the strength of agreement was weak, the working group aimed to revise the statements as appropriate and if this could be achieved with agreement of the working group then the revised statements were re-circulated for a further round of the Delphi process. Any questions arising from this process were resolved by face-to-face meetings or virtual communications between group members and the core committee. The consensus guideline manuscript was then discussed at further milestone meetings and iterations were circulated electronically. The document was then finalised and circulated to all authors for final approval.

5.4 Results

An overview of all the groups statements, grading of evidence and consensus voting outcomes are summarised in table 12.

Table 12. Summary of statements, level of evidence, recommendations, and level of agreement

Question	Statement	Quality assessment	Recommen-dation	Agreement
Guidelines for the understanding and management of pain in chronic pancreatitis (122)				
What is the natural history and burden of pain in CP (in relation to treatment)?	Abdominal pain is the most frequent symptom of CP. However, the severity, temporal nature, and natural history of pain is highly variable	Moderate	Strong	Strong 1.0
Are there different types of pain in CP	Pain in CP remains poorly understood and inadequately correlated with neurobiological mechanisms. CP is characterized by inflammation but unlike other inflammatory disorders, there is a paucity of therapeutic attempts targeting this particular aspect of pathophysiology. On the other hand, there are striking changes in structure and function in both the peripheral and central nervous system in this condition, lending plausibility to a maladaptive state that includes both neuropathic and dysfunctional pain. In the absence of effective anti-inflammatory approaches, it is clearly important to focus on the alteration of function that accompanies these changes in the nociceptive system as a potential therapeutic target.	Low	Strong	Strong 0.92
Which methods are available to assess pancreatic pain and its response to treatment?	Assessment of pain in CP follows the guidelines for other types of chronic pain, where the multidimensional nature of symptom presentation is taken into consideration. Only a few instruments have been validated for subjective pain assessment in CP; however, several appropriate measures exist despite not being rigorously validated in this population.	Moderate	Strong	Strong 0.92
What is the role of smoking and alcohol on pain treatment in CP?	Abstinence from alcohol and smoking, in addition to adequate treatment, should be strongly advised in patients with CP.	Moderate (alcohol) Weak (smoking)	Strong	Strong 1.0
Do enzymes and antioxidants influence pain in CP?	Pancreatic enzyme therapy with high protease content may be tried as an initial treatment for pain relief in patients with CP. Furthermore; combination of antioxidants in sufficient dosages should be included in the armamentarium of pain treatments.	Moderate	Strong	Weak 0.64
Which analgesics are recommended for pain in chronic pancreatitis?	Currently the standard guideline for analgesic therapy in CP follows the principles of the “pain relief ladder” provided by the World Health Organization (WHO) adjusted to the pain characteristics of this condition.	Moderate	Strong	Strong 0.92

Is endoscopic therapy effective for pain treatment in CP?	The best candidates for successful treatment of painful CP with first-line endoscopic therapy are patients with distal obstruction of the main pancreatic duct (single stone and/or single stricture in the head of the pancreas) and in the early stage of the disease. Endo- scopic therapy can be combined with Extracorporeal Shock Wave Lithotripsy (ESWL) in the presence of large (>4 mm) obstructive stone(s) located in the pancreatic head, and with ductal stenting in the presence of a dominant main pancreatic duct stricture that induces a markedly dilated duct. (Quality assessment: moderate; Recommendation: strong; Agreement: conditional)	Moderate	Strong	Conditional 0.73
Is ESWL effective for pain treatment in CP?	In patients with uncomplicated painful calcified CP, ESWL alone is a safe and effective treatment. Best candidates for benefiting from initial first-line ESWL are patients with obstructive calcifications, > 4 mm confined to the head of pancreas. Combining systematic endoscopical therapy with ESWL adds to the cost of patient care, at the same time not probably improving the outcome of pancreatic pain (Quality assessment: moderate, Recommendation strong; Agreement: conditional).	Moderate	Strong	Conditional 0.73
Are other treatments (psychological, neurolytical etc.) of value for pain treatment in CP?	Neurolytical interventions can be used in selected patients with painful CP who have failed endoscopic and surgical treatment. Thoracoscopic splanchnic denervation is more effective regarding long-term pain relief in patients who are not on chronic opioid treatment. Behavioral interventions should be part of the multi- disciplinary approach in CP pain particularly when patients experience psychological impact of pain and quality of life has decreased. Early intervention in children may be particularly important.	Low	Strong	Conditional 0.73
What is the optimal surgical approach to release pain in CP?	Depending on the morphological changes of the pancreas and pain processing status a (partially) resection, decompression of the pancreatic duct or combined interventions can be performed to reduce pain. Long-term effects are variable, but success rates up to 80% have been reported. The emerging role of total pancreatectomy as initial surgical treatment looks promising but needs further investigation (Quality assessment: moderate; Recommendation: strong; Agreement: conditional)	Moderate	Strong	Conditional 0.73
When is the optimal time for surgery in painful CP?	Current evidence on the timing of surgery for painful CP suggests a beneficial role for early surgery, i.e. 1) within the first 2e3 years after	Low	Weak	Strong 0.83

	diagnosis or symptom onset, 2) for patients who had equal to or fewer than 5 endoscopic procedures, and 3) for patients who have not yet required opioid analgesics for medical pain treatment.			
How to manage pain "relapse" after surgery or endoscopy?	Current evidence suggests that the first step for the management of pain relapse should be exclusion of obstructing stones or strictured anastomosis via imaging, followed by a limited number endoscopic interventions, and early consideration of re-surgery to achieve pain control.	Weak	Strong	Weak 0.64
What are the indications for referral to a specialist centre for further investigation of pain?	All patients with presumed or established diagnosis of CP should be routinely referred to specialist pancreatic centres for investigation and treatment of their disease.	Moderate	Strong	Strong 1.0
International consensus statements on early chronic Pancreatitis (163)				
What is Early Chronic Pancreatitis?	The term Early Chronic Pancreatitis describes the initial stage of definite chronic pancreatitis.	Low	Conditional	Conditional 0.77
What does the word “Early” mean in the definition of Early Chronic Pancreatitis.	The word “Early” in early chronic pancreatitis is used to describe disease state, not disease duration.	Moderate	Strong	Strong 1.0
What does the word “chronic” mean in the definition of Early Chronic Pancreatitis?	The word “Chronic” in early chronic pancreatitis is used to describe disease character and duration.	Moderate	Strong	Conditional 0.69
How does Early Chronic Pancreatitis affect pancreatic function?	Early chronic pancreatitis defines a stage of CP with pre- served pancreatic function and potentially reversible features.	Low	Strong	Strong 0.83
Can Early Chronic Pancreatitis be diagnosed by abdominal imaging techniques alone?	Early Chronic Pancreatitis cannot be diagnosed based on currently available imaging techniques alone.	Moderate	Strong	Conditional 0.77
Can Early Chronic Pancreatitis be diagnosed by a com- bination of factors?	Theoretically Early CP can be diagnosed based on a combination of (a) the presence of high risk factors for CP, (b) low risk for other disorders with features that overlap CP, (c) appropriate clinical context and (d) supportive biomarkers	Low	Strong	Weak 0.62

Is acute pancreatitis a mandatory risk factor for Early Chronic Pancreatitis?	A history of acute pancreatitis, and especially recurrent acute pancreatitis, are significant risk factors for early chronic pancreatitis, but are not mandatory to make a diagnosis.	Low	Conditional	Weak 0.62
Are genetic variants a required risk factor for Early Chronic Pancreatitis?	Genetic variants are important risk factors for early chronic pancreatitis, but they are neither necessary nor sufficient to make a diagnosis.	Moderate	Strong	Strong 0.85
Are environmental factors required risk factors for Early Chronic Pancreatitis?	Environmental risk factors can provide important evidence in favor of early chronic pancreatitis, but they are neither necessary nor sufficient to make a diagnosis.	Moderate	Strong	Strong 0.92
What is the differential diagnosis for Early Chronic Pancreatitis?	The differential diagnosis for Early CP includes any other disorder with features that overlap the features of chronic pancreatitis as defined in the Mechanistic Definition of chronic pancreatitis.	Moderate	Strong	Strong 0.83
Guidelines for the Diagnostic Cross Sectional Imaging and Severity Scoring of Chronic Pancreatitis (90)				
What are the indications for CT in the investigation of CP?	CT is indicated as part of a diagnostic algorithm when there is clinical suspicion of CP, in the presence of typical symptoms and recognized risk factors. CT is also indicated to exclude other potential intraabdominal pathologies presenting with symptoms like CP. In patients with established CP, CT is indicated to assess complications and the need for further interventions.	High	Strong, 1A	Strong 1.0
Is CT the best initial test when investigating CP, and should CT be performed as a baseline investigation in all CP patients?	CT is the best initial imaging modality for the evaluation of patients with suspected CP, because it is widely available and can depict most changes in pancreatic morphology (parenchymal atrophy, parenchymal or ductal calcifications, ductal changes and complications). CT is also useful to detect incidental lesions and pathology of the pancreas, e.g. malignancy or autoimmune aetiology.	Moderate	Strong, 1B	Strong 1.0
Can a normal CT exclude CP, and can early or mild CP be diagnosed on CT?	Despite CT being the imaging modality of choice for initial investigation of CP, it cannot exclude a diagnosis of CP nor can it be used to exclusively diagnose early or mild CP.	High	Strong, 1A	Strong 1.0
What are the indications of MRI/MRCP in the investigation of CP?	MRI/MRCP is indicated in the investigation of CP, especially in patients where no specific pathological changes are seen on CT, but the clinical suspicion of a diagnosis remains high. MRI/MRCP is superior to CT in identifying early CP changes or mild degrees of CP.	Moderate	Strong, 1B	Strong 1.0
Can the ERCP Cambridge criteria (1984) for CP be	Although the Cambridge Classification system cannot be directly translated to MRCP findings and ERCP tends to overestimate of the	Moderate	Strong, 1B	Strong 0.83

extrapolated to MRCP findings?	calibre of the MPD, a very good correlation has been described be- tween ERCP and MRCP findings. However, standard MRCP (without secretin administration) has low sensitivity in diagnosing mild CP since very subtle ductal changes cannot be clearly identified.			
Should secretin-stimulated MRCP be used in the investigation and diagnosis of CP?	In the depiction of subtle ductal changes, secretin-stimulated MRCP is more accurate than standard MRCP, and should after a negative MRCP be considered when there is clinical suspicion of CP.	Moderate	Weak, 2B	Conditional 0.75
Can a normal MRCP exclude a diagnosis of CP, and can early or mild CP be diagnosed on MRI?	A normal MRI/MRCP without secretin-stimulation cannot exclude the diagnosis of early/mild CP where the ductal changes are very subtle. In these cases, s-MRCP (or EUS) should be considered although early changes still cannot be excluded.	Moderate	Strong, 1B	Strong 0.92
When is EUS needed (in addition to cross sectional imaging) in the diagnosis and grading of CP?	EUS is considered to be the most appropriate and sensitive imaging technique to diagnose parenchymal and ductal changes, mainly during the early stage of the disease. Hence EUS is indicated when CT (and MRI) are negative or doubtful in patients with clinical suspicion of CP.	High	Strong, 1A	Conditional 0.75
Are there any validated radiological severity scoring systems for CP?	No validated radiological severity scoring systems for CP are available, although a modified Cambridge Classification as used for ERCP has been used for MRCP.	High	Strong, 1A	Strong 1.0
Is there a need for CT/MRI based criteria to assess the severity of CP?	There is an unmet need for development of a new and validated radiological scoring system based on imaging criteria for the assessment of CP severity.	High	Strong, 1A	Strong 1.0
How can imaging currently used in clinical practice be utilized in a scoring system of CP severity?	CT and MRI complement each other in depicting the pathological changes seen with CP including glandular volume loss, ductal changes, parenchymal calcifications and parenchymal fibrosis. Secretin stimulated MRCP in addition, can provide assessment of exocrine function and ductal compliance. These imaging parameters can then be incorporated together with clinical findings in the clinical classification and severity grading of CP.	Moderate	Weak, 2B	Strong 0.92
What criteria should be considered as vital for inclusion in a radiological severity scoring system for CP?	Grading of gland atrophy, ductal changes, parenchymal calcifica- tions and gland fibrosis should be included in a radiological severity scoring system. Quantification of exocrine function can be included as supplementary information.	Moderate	Strong, 1B	Strong 1.0

How should the severity of CP be graded by CT and MRI?	Severity grading by CT and MRI/MRCP should include ductal changes, parenchymal changes (calcifications on CT and fibrosis on MRI), gland atrophy and extent of pancreatic involvement. Assessment of exocrine function based on secretin-MRCP could also be factored in to the grading. (Quality assessment: Low; Strength of recommendation: Weak; Grade 2C; Agreement: Conditional (alpha-score 75%))	Low	Weak, 2C	Conditional 0.75
International consensus guidelines for surgery and the timing of intervention in chronic pancreatitis (107)				
What are the indications for surgery in CP?	The most common indication for surgery for CP is intractable pain.	High	Strong	Strong 0.86
	Other indications for surgery are a suspicion of neoplasm.	High	Strong	Strong 1.0
	Other indications for surgery are local complications of adjacent organs, such as duodenal or common bile duct stenosis, pseudoaneurysm or erosion of the large vessels, large pancreatic pseudocysts and internal pancreatic fistula.	Moderate	Conditional	Strong 0.86
What is the optimal timing of surgery?	Surgery early in the disease process of CP is favoured over surgery in a more advanced stage of disease to achieve optimal long-term pain relief.	Moderate	Strong	Strong 1.0
	The risk of developing pancreatic exocrine insufficiency is lower after early surgery for CP than after surgery performed in an advanced disease stage. Pancreatic resection techniques have a higher risk for PEI than drainage techniques.	Low	Conditional	Conditional 0.79
	No recommendation can be drawn from the evidence regarding the effect of early surgery on developing endocrine pancreatic function during follow-up due to few and contradicting studies.	Low	None	Weak 0.64
	Long-term quality of life is improved after early surgery (<3 years of onset) compared to surgery in a more advanced stage of disease.	Low	Conditional	Conditional 0.79
What are the surgical options for the treatment for CP? What operative technique should be used for patients with CP and enlarged pancreatic head?	In patients with CP and an enlarged pancreatic head, performing a combined drainage and resection, such as Frey, Beger, and Berne procedure may be the treatment of choice. The combined procedure such as Frey or Beger procedure have been shown to be superior to pancreaticoduodenectomy (PD) or pylorus-preserving pancreaticoduodenectomy (PPPD) in terms of postoperative complications; with comparable results in pain relief results. The Frey and Berne procedures have similar results when compared to each other and to the Beger procedure from which they are both derived. The Frey and	High	Strong	Strong 0.86

	Berne procedures have however a lower morbidity rate with a comparable effect on pain control and quality of life.			
What operative technique should be used for patients with CP and a dilated pancreatic main duct and a documented normal size pancreatic head?	In adult patients, a main duct diameter of 5 mm or more in the pancreatic body seems amenable to ductal drainage for the majority of pancreatic surgeons. This threshold of 5 mm could be proposed as definition of a “dilated main duct”.	Low	Conditional	Conditional 0.71
	Both, the extended lateral pancreaticojejunostomy on a Roux-en-Y loop and Frey procedure seems to provide equivalent pain control in patients with main duct dilatation and normal size pancreatic head, but studies with a direct comparison of the two techniques are lacking. For patient with painful CP and a dilated duct and normal size pancreatic head, an extended lateral pancreaticojejunostomy on a Roux-Y loop and Frey procedure provide comparable pain control (low quality of evidence). No preference can be made which is the best surgical technique of the two in these patients.	Low	Conditional	Weak 0.5
What is the role of surgery in groove pancreatitis?	Surgery should be performed when medical and endoscopic options have failed. Surgery should be aimed at pain relief and/or complete pain resolution, and to solve the malnutrition status	Very low	Conditional	Conditional 0.71
	Initial treatment of groove pancreatitis should involve medical treatment and occasionally, endoscopic drainage procedures may be helpful. If these approaches fail, the patient should be referred for surgery.	Very low	Conditional	Conditional 0.71
	In expert hands, pancreaticoduodenectomy is the most suitable surgical option for patients with groove pancreatitis.	Low	Conditional	Strong 0.86
How to assess the risk of pancreatic cancer in a patient with CP? Is there a role for prophylactic (cancer) surgery?	Surgical resection should be chosen for a suspected malignant cystic lesion.	High	Strong	Strong 1.0
	The risk of pancreatic carcinoma is somewhat higher in patients with CP but still too low to recommend active screening or prophylactic surgery.	Moderate	Conditional	Strong 0.93
	Patients with hereditary CP have such a high risk of pancreatic cancer that prophylactic resection can be considered.	Moderate	Conditional	Weak 0.57

International Consensus Guidelines for Risk Factors in Chronic Pancreatitis (164)				
What are the risk factors for CP?	Alcohol, smoking, and certain genetic alterations are risk factors for CP.	High	Strong	Strong 1.0
What should be done to determine the risk factors/aetiology for CP at the time of diagnosis?	Past history, family history, onset of symptoms, and life-style factors including alcohol intake and smoking history should be determined.	High	Strong	Strong 1.0
	Laboratory data including serum triglycerides, calcium, IgG4, and possible morphologic abnormalities of the pancreas including pancreas divisum might be assessed.	Low	Weak	Strong 0.89
	In idiopathic disease, full sequence analysis of the CFTR, CPA1, CTRC, PRSS1 and SPINK1 gene exons and exon-intron boundaries and testing for the CEL gene pathogenic hybrid allele is recommended in order to explore the genetic background.	Low	Conditional	Conditional 0.74
How much alcohol consumption can be considered as risk factor/aetiology for CP?	Statement-5. Alcohol consumption dose-dependently elevates the risk of CP. Heavy drinkers have some 5 times more chances to develop CP than non-alcohol consumers.	Moderate	Strong	Strong 1.0
	Alcohol consumption of less than 60 g/day in-creases the risk and promotes the progression of CP in susceptible individuals.	Low	Conditional	Weak 0.63
	Statement-7. Alcohol consumption of equal to or more than 60 g/day increases the risk of CP.	Moderate	Strong	Strong 1.0
	The effect of alcohol seems to be independent of smoking.	Low	Conditional	Conditional 73.7
	Alcohol abuse increases the risk of progression from acute pancreatitis to CP. After an acute attack of pancreatitis almost half of alcohol abusers develop CP. Figures rise to 80% after recurrent pancreatitis.	Moderate	Strong	Strong 0.895
How much smoking can be considered as risk factor/aetiology for CP?	Ever smokers (even smoking less than 1 pack of cigarettes per day) have an increased risk for CP, as compared to never smokers.	Moderate	Strong	Strong 0.895
	There seems to be a dose-response effect for the amount of daily consumption on the risk to develop CP.	Low	Conditional	Strong 0.947
	Risk increases with time of exposure.	Low	Conditional	Strong 0.895

	Risk tends to diminish with abstinence (former smokers).	Low	Conditional	Strong 0.947
	Risk seems to be independent of alcohol abuse.	Low	Conditional	Conditional 0.79
The role of total pancreatectomy with islet autotransplantation in the treatment of chronic pancreatitis (165)				
What are the outcomes of TPIAT for patients with CP?	Improvement in quality of life is attained in patients with chronic pancreatitis following TPIAT.	Moderate	Strong	Strong 0.94
	Pancreatic pain and opioid use are significantly reduced in patients with chronic pancreatitis following TPIAT.	Moderate	Strong	Strong 1.0
	TPIAT may be associated with reduced medical utilization after the operation, however, evidence is currently limited for healthcare utilization following TPIAT.	Low	Weak	Strong 0.81
When should TPIAT be considered for CP versus other therapy forms (e.g. continued medical care, endoscopic therapies, other surgeries, i.e. head resection or drainage)?	The true standing of total pancreatectomy with islet auto-transplantation (TPIAT) among all forms of CP therapy is not yet identified. Studies including head-to-head comparisons of TPIAT with other therapy options such as medical, endoscopic or other surgery forms (e.g. DPPHR or ppWhipple) are lacking.	Low	Weak	Conditional 0.75
What are the unique benefits of TPIAT over TP alone?	TPIAT offers the possibility of insulin independence and seems to be superior to TP alone in glycemic control and long-term diabetes outcome. The option of TPIAT should be considered and offered to CP patients requiring total pancreatectomy.	Low	Weak	Strong 0.94
What are the indications and contraindications for TPIAT?	The main indication for TPIAT is debilitating pain from CP, or recurrent pancreatitis that limits the subject's quality of life.	Low	Strong	Strong 0.94
	The major contraindications include but are not limited to: active alcoholism, the presence of pancreatic cancer, end-stage systemic illness, a psychiatric or socioeconomic status that precludes the performance of the surgery and the care afterwards safely.	Low	Strong	Strong 0.94
What factors are associated with favorable or poor pain outcomes after TPIAT for CP?	TPIAT might be considered for effective management in well-defined, selected cohorts of CP patients. Early surgery, i.e. at a young age, before multiple endoscopic attempts and prior to activation of neuropathic pain circuits, is likely to enable better pain outcomes.	Low	Weak	Conditional 0.69

What factors are associated with favorable or poor diabetes mellitus outcomes?	slet mass transplanted is the variable most consistently predictive of islet graft function and insulin independence across multiple studies. Prior pancreatic surgery, advanced pancreatic disease including calcifications, alcoholic pancreatitis, and possibly prolonged disease might adversely impact islet mass or chance of insulin independence.	Low	Weak	Strong 0.88
Guidelines on the histopathology of chronic pancreatitis (140)				
What are the classical histopathological features of CP?	The cardinal features of CP are the triad of fibrosis, loss of acinar tissue (atrophy) and duct changes (distortion and dilatation).	Moderate	Conditional	Strong 0.9
	Other features include pseudocysts, islet aggregation, more prominent and enlarged peripheral nerves, fibrous thickening and obliteration of blood vessels, and squamous metaplasia within duct epithelium.	Moderate	Conditional	Weak 0.6
Are there histopathological features that distinguish the aetiology of CP?	There are no unique histopathological features that distinguish the different aetiologies of CP.	Moderate	Conditional	Strong 0.8
Do histopathologists agree on which morphological features are required to make a histological diagnosis of CP?	Clinical history and investigations are most important in establishing the aetiology.	Moderate	Strong	Strong 1.0
	Histopathologists do not agree on what features are required to make a histological diagnosis of CP.	Moderate	Conditional	Weak 0.6
How do histopathologists assess the severity of CP?	There is no reproducible and universally accepted histological grading system for assessing the severity of CP.	None	Conditional	Strong 0.8
	A classification of “mild”, “moderate” and “severe” is usually used but not validated using histopathological features.	Moderate	Conditional	Strong 0.9
What is the significance of focal or asymptomatic fibrosis?	There are scoring systems for fibrosis that are used as research tools but are not validated for clinical use.	Low	Strong	Strong 1.0
	Asymptomatic fibrosis is a common finding associated with ageing and is not necessarily a feature of CP.	Low	Strong	Strong 0.8
What is early CP on histopathology?	Histopathological assessment is usually done in advanced disease, when resected pancreatic tissue is available, so that information about the histopathology of early CP is limited.	Low	Strong	Strong 0.9

	There are usually no obvious or diagnostic macroscopic features of early CP.	Low	Strong	Strong 0.8
	Interlobular fibrosis, distortion of interlobular ducts, presence of chronic inflammatory cells and resolving focal fat necrosis may be observed microscopically.	Low	Conditional	Strong 0.9
Is histopathology the gold standard for diagnosing CP?	At present, histopathology is not the gold standard for the diagnosis of CP, and the pathologist should also take into account the clinical history and radiological features of the pancreas in making the diagnosis.	Moderate	Conditional	Strong 0.9
	EUS-guided fine needle and core biopsies are useful to differentiate chronic pancreatitis from pancreatic neoplasia or autoimmune pancreatitis.	Moderate	Conditional	Conditional 0.7
	Cytology alone is not a reliable method for the diagnosis of chronic pancreatitis.	low	Strong	Strong 1.0
International consensus guidelines on surveillance for pancreatic cancer in chronic pancreatitis (166)				
Should all patients with chronic pancreatitis undergo screening or surveillance for PDAC?	The prevalence of pancreatic cancer in sporadic chronic pancreatitis is not high enough to justify screening or screening	High	Conditional	Conditional 0.74
	The risk of pancreatic cancer in affected individuals with an autosomal dominant history of hereditary pancreatitis due to inherited PRSS1 mutations is high enough to justify surveillance.	High	Strong	Strong 0.83
	The risk of pancreatic cancer in affected individuals with an autosomal dominant history of hereditary pancreatitis but without PRSS1 mutations is high enough to justify surveillance.	Moderate	Weak	Weak 0.57
	The risk of pancreatic cancer in patients with chronic pancreatitis associated with SPINK1 p. N34S is not high enough to justify screening or surveillance.	Moderate	Strong	Strong 0.8
	The risk of pancreatic cancer in patients with chronic pancreatitis associated with other germline mutations including those of CFTR, CTRC, CPA1, and CEL, is not high enough to justify screening or surveillance.	Moderate	Conditional	Conditional 0.7
What are the best available surveillance methods?	The best available surveillance methods are CT and MRI.	Weak	Conditional	Conditional 0.7
	EUS should not be used for surveillance as early tumours may be obscured by inflammation, fibrosis and calcification.	Moderate	Weak	Weak 0.52

Where should surveillance for PDAC in patients with HP due to PRSS1 mutations be undertaken?	Surveillance for pancreatic cancer in patients with hereditary pancreatitis due to PRSS1 mutations should be undertaken in pancreatic specialist centers.	Low	Strong	Strong 1.0
When should surveillance be initiated and stopped in HP?	Surveillance should only be introduced after the age of 40 years and stopped when the patient would no longer be suitable for surgical intervention.	Moderate	Strong	Strong 0.97
What advice should patients with CP be given in managing their disease in order to reduce risk of developing PDAC?	Patients should be advised to avoid use of to- bacco, not drink alcohol, have a balanced healthy diet containing daily fruit and vegetables with a high folate intake, whilst moderating the intake of red meat and taking some form of regular high physical exercise, altogether aiming to avoid obesity.	Moderate	Strong	Strong 0.91
International consensus guidelines on the role of diagnostic endoscopic ultrasound in the management of chronic pancreatitis (101)				
What should the EUS-criteria be for a firm diagnosis of chronic pancreatitis.	The ideal threshold number of EUS criteria necessary to diagnose chronic pancreatitis has not been firmly established, but the presence of 5 or more and 2 or less strongly suggests or refutes the diagnosis, respectively. The Rosemont scoring system standardizes the reporting of EUS signs indicative of chronic pancreatitis, but further studies are needed to demonstrate that it contributes to an overall improvement of the diagnostic accuracy over conventional scoring.	Moderate	Strong	Strong 1.0
Is the diagnosis of chronic pancreatitis by scoring EUS-criteria reliable?	Specificity, inter- and intra-observer variability and pre-test probability limit the reliability and utility of EUS to diagnose chronic pancreatitis and in particular early stages of the disease.	Moderate	Strong	Strong 0.83
International consensus guidelines on interventional endoscopy in chronic pancreatitis (167)				
What are the indications for intervention in chronic pancreatitis	Endoscopic or surgical treatment should be offered to patients with chronic pancreatitis with persistent severe pain. Intervention in the form of either surgery or endotherapy is not recommended in asymptomatic patients with chronic pancreatitis who do not have abdominal pain to improve pancreatic exocrine and/or endocrine function or prevent cancer.	Moderate	Strong	Strong 1.0
	Endoscopic or surgical treatment should be carried out after careful patient selection for local complications of chronic pancreatitis with persistent clinical symptoms such as gastric outlet obstruction, duodenal stenosis, biliary obstruction with cholestasis and pseudocysts.	Moderate	Strong	Strong 1.0

	Celiac plexus block may be undertaken in patients for significant abdominal pain who are not candidates for pancreatic surgery, or have not responded to endotherapy and extracorporeal shock wave lithotripsy (ESWL), or have a poor general condition as a temporizing measure before definitive therapy.	Moderate	Weak	Weak 0.41
What is the best strategy for the treatment of pancreatic ductal stricture?	Non-surgical decompression of the main pancreatic duct including endoscopic therapy can be selected for immediate pain relief of chronic pancreatitis before considering surgery. Surgical intervention should be considered if endoscopic procedure fails or has temporary success needing repeated endoscopic therapy.	Moderate	Conditional	Strong 0.88
	If there are contraindications for surgical therapy in patients in whom conventional endoscopic therapy has failed, endosonographic-guided drainage of the pancreatic duct is another option for pain control.	Moderate	Weak	Weak 0.47
	Endoscopic drainage should be the preferred modality for treating pancreatic pain and biliary stricture in patients with chronic pancreatitis who have associated portal/splenic vein thrombosis.	Moderate	Conditional	Strong 0.82
How should endoscopic stent treatment be done for pancreatic ductal stricture. Statement?	A straight plastic pancreatic stent should be placed across the stricture depending on the caliber of the stricture of the pancreatic duct.	Low	Conditional	Strong 0.88
	An endoscopic retrograde cholangiopancreatography (ERCP) inserted endoscopic stent should be removed or exchanged at between 2 and 3 months later. At this time a new stent should be inserted if there is still a significant stricture.	Low	Conditional	Strong 0.88
What is the strategy for the treatment of pancreatic ductal stones.	ESWL should be the first-line therapy as non-surgical intervention for main pancreatic duct stones in patients with chronic pancreatitis who do not get adequate pain relief with conservative management although a stent placement may be done first to relieve pain.	Low	Conditional	Conditional 0.71
	Endoscopic extraction is indicated for small stones or stone fragments after ESWL.	Moderate	Conditional	Strong 0.82
What is the strategy for the treatment of pancreatic pseudocysts in chronic pancreatitis. Statement	For pancreatic pseudocysts that cause symptoms and/or complications, interventional or surgical treatment should be performed.	Moderate	Strong	Strong 0.94

	Underlying stricture or disruption of the main pancreatic duct with symptoms and/or complications should be treated with endoscopic transpapillary placement of a pancreatic stent for pseudocysts <5cm and with communication with the main pancreatic duct.	Moderate	Conditional	Conditional 0.71
	symptomatic and uncomplicated pancreatic pseudocysts of more than 5 cm in diameter that do not resolve within six weeks may be treated with transmural drainage.	Low	Weak	Weak 0.53
	In case of a suspected neoplastic cystic lesion diagnostic needle aspiration of the cyst may be done.	Low	Weak	Weak 0.53
	urgical intervention should be considered if endoscopic drainage of pseudocysts fails or has temporary success needing repeated endoscopic therapy, especially when there is disconnected duct syndrome, inflammatory mass, and intraductal calculi with duct strictures.	Low	Conditional	Strong 0.82
What is the strategy for the treatment of distal main biliary duct obstruction in chronic pancreatitis.	Endoscopic treatment is recommended when the patients show symptoms related to the distal bile duct obstruction (obstructive jaundice and/or acute cholangitis), and in persistent cholestasis with alkaline phosphatase elevation (>2-3 times) for at least month even in asymptomatic patients.	Low	Conditional	Strong 0.88
	Endoscopic treatment with multiple plastic or covered metal stents may be effective for relieving of the symptoms related to the distal bile duct obstruction due to chronic pancreatitis.	Low	Weak	Conditional 0.71
	In main biliary duct strictures caused by chronic pancreatitis, biliary stent placement is recommended for a period of 6 months to 1 year.	Low	Weak	Weak 0.65
	Plastic stent replacement for main biliary duct stricture is recommended every 3 months. The optimal period for replacement of covered metal stent is currently unknown.	Low	Weak	Conditional 0.71
	Endoscopic placement of multiple plastic or covered metal stent and/or surgery are appropriate to manage refractory bile duct obstruction.	Low	Conditional	Conditional 0.76
	Surgical treatment should be planned if bile duct obstruction reoccurs after one year of endoscopic stent treatment. For the patients who have significant calcifications and/or mass of the pancreatic head, surgical treatment may be preferred as an initial treatment.	Low	Strong	Strong 0.94
What is the strategy for the treatment of internal pancreatic fistula and	Endoscopic interventional therapy should be undertaken for the management of internal pancreatic fistula in patients presenting with main pancreatic duct disruption or obstruction.	Low	Conditional	Strong 0.88

pancreatic pleural effusion and ascites in chronic pancreatitis.				
What is the strategy for the treatment of hemosuccus pancreaticus in chronic pancreatitis.	Percutaneous endovascular treatment should be the first choice of treatment for hemosuccus pancreaticus in hemodynamically stable patients. However, patients with hemodynamic instability and unsuccessful embolization should undergo surgery.	Low	Conditional	Strong 0.94
What is the strategy for the treatment of duodenal obstruction in chronic pancreatitis.	Surgical treatment is recommended for duodenal stenosis associated with chronic pancreatitis, as endoscopic treatment is difficult in such cases.	Moderate	Conditional	Strong 0.88
	Duodenal stenosis due to chronic pancreatitis should be carefully differentiated from pancreatic cancer.	Moderate	Strong	Strong 1.0

5.5 Discussion

The ICGCP process was successful in achieving the goals established by the founders back in 2016. Data and consensus results collected and analysed during the modified Delphi process have been published in peer reviewed journals with a global target audience. Reaching consensus and gaining international adoption of the mechanistic definition of CP (10) was key to initiating and advancing this project by encouraging a paradigm shift in the approach to addressing CP related challenges. The ICGCP differed from previously published CP guidelines, and they were the first truly international and multidisciplinary set of guidelines.

Strong consensus was achieved in many areas in each subgroup. Areas which only achieved weak consensus have helped to highlight where the current controversies still lie and given focus to areas for future work. These areas mainly related to the definition and diagnosis of early CP, although significant groundwork was made.

There was consensus agreement over the meaning of the word “early” when defining early CP, relating to disease characteristics opposed to disease duration. Early CP was also agreed to be a stage of the disease process where pancreatic function is preserved, and features may be reversible (163).

Risk factors for CP were addressed from differing viewpoints by several of the working groups including the early CP group, pain management, risk factors for CP group and the screening in CP group. There was strong consensus across these groups relating to the importance of genetic and environmental aetiologies in the development and the progression of CP. Consensus was to counsel patients to avoid any form of tobacco use and to avoid/exclude all alcohol consumption and in certain cases genetic counselling should be applied to reduce the incidence of CP.

CT cross sectional imaging was the imaging modality of choice for the initial and follow up investigation of all patients with suspected CP. However, there

was strong agreement that CT cannot diagnose early CP, nor can it exclude a diagnosis of CP. MRI/MRCP was agreed to be superior to CT in identifying early CP changes or mild degrees of CP, but again a normal MRI could not exclude a diagnosis of CP. There was strong agreement that there is currently an unmet need for an internationally accepted and validated CP radiology scoring system based on imaging criteria including glandular volume loss, ductal changes, parenchymal calcifications, parenchymal fibrosis, and exocrine function based on CT and MRI (90).

Regarding EUS examination, there was consensus that the number of EUS criteria needed to meet a threshold to diagnose CP was currently not known and that the Rosemont scoring system did not provide any diagnostic benefit. There was also strong agreement that intra-observer variability as well as pre-test probability which may introduce interpreter bias and limits the diagnostic accuracy of EUS. This aspect of the guideline work identified several factors that may negatively impact on the diagnostic specificity of EUS in CP including effect of age, smoking, diabetes, and steatosis for example which can result in false positive diagnoses (101). There is an urgent need for further research in this area.

International histopathology experts strongly agreed that the cardinal features of CP are the triad of fibrosis, loss of acinar tissue and duct changes however, histopathology is ***not*** the gold standard for the diagnosis of CP (140). Histopathology is unable to differential between the changes associated with other processes such as age-related changes, smoking, alcohol etc and histopathology cannot differentiate between different aetiologies of CP. The lack of a reproducible and universally accepted histological grading system for assessing the severity of CP was highlighted along with the paucity of diagnostic macroscopic features of early CP.

Both the surgery in CP and the interventional endoscopy in CP groups reached consensus on timings and indications for interventions. Early surgery was favoured over interventional endoscopy in advanced CP as surgery is most likely to achieve successful long term pain relief (107).

Due to weak agreement on the use of celiac plexus block in the management of pain in CP and significant concerns about clinical sequalae including inflammation and fibrosis, both the pain management groups, and the Interventional endoscopy group suggested the abandonment of this procedure for this indication at present (167).

There was strong consensus that total pancreatectomy with auto-transplantation (TPIAT) can improve QoL and reduce opiate dosing in well selected patients and that TPAIT is preferred over total pancreatectomy where feasible. The challenges and controversies surrounding patient selection and identifying the 'best' candidate for surgery were highlighted (165). There are currently no studies available which allow direct comparison of TPAIT to other pancreatic surgeries of endoscopic treatments.

Regarding PDAC surveillance in CP, there was strong consensus that individuals with a proven PRSS1 mutation should be screened, that this screening should only be undertaken in appropriate specialist pancreatic centres, and that screening should be initiated after 40 years of age and stopped when that individual is no longer able to receive surgical intervention. Patients with hereditary CP have such a high risk of pancreatic cancer that prophylactic resection can be considered. Those with a SPINK-1 mutation do not have sufficiently high enough risk of PDAC to warrant screening. There was ongoing debate regarding the most appropriate imaging modality for on-going screening in HP patients (166).

The diagnosis of early CP remained a challenging and controversial topic. The concept of early CP is well established, but there are no established definitions or diagnostic criteria. Within the ICGCP expert group, there was only weak agreement that early CP can be diagnosed based on a combination of factors including risk factors for CP, low risk for alternative diagnoses with features like CP, appropriate clinical context, and supportive biomarkers. The role and relevance of acute and recurrent acute pancreatitis as risk factors for the development of early CP and CP were also unclear. New approaches to the accurate diagnosis of early CP may require a definition that considers genetic

and environmental risk factors, biomarkers, clinical context (including history of acute pancreatitis and imaging) and new models of disease, requiring prospective validation.

5.6 Conclusion

The ICGCP process has made significant progress in bringing a truly international spotlight on to the clinical diagnosis and management of early CP and CP. The international pancreatology community can use these guidelines to assist consolidating clinical practice based on recommendations with strong agreement. Areas with weak agreement should have practices reviewed, curtailed, or modified as appropriate until further evidence is available to guide future recommendations.

The clear unmet need for standardised and validation scoring systems in several areas including early CP, radiology, EUS examination, and histopathology have been brought to global attention and resulted in several new international research initiatives. Importantly, both genetic and environmental factors were found to play crucial roles in the development of CP. Therefore, health-promoting lifestyle education and in certain cases genetic counselling should be applied to reduce the incidence of CP.

The challenges still faced around the definition and diagnosis of early CP have been highlighted. The mechanistic definition of CP will help facilitate progress in establishing a formal definition of early CP. The definition and diagnosis of Early CP cannot be dependent on morphological changes and instead should focus on the underlying factors that drive disease progression and establishing potential early therapeutic targets. The early CP problem requires a precision medicine approach to allow more accurate individualised diagnoses, treatment plans, and to also encourage further prospective research.

Chapter 6: The EUROPAC disease registry and secondary screening study: Hereditary Pancreatitis arm

6.1 Introduction

The European Registry of Hereditary Pancreatitis and Familial Pancreatic Cancer (EUROPAC) is the largest registry of hereditary pancreatic diseases in Europe, with the main source of recruitment being Western Europe.

6.1.1 The purpose of disease registries

Disease registries are designed with respect to their intended purpose. Traditionally registries have 4 main purposes which can be described in terms of patient outcomes (1) describing the natural history of disease, (2) determining clinical and/or cost-effectiveness, (3) assessing safety or harm, and (4) measuring or improving quality of care. Additionally one should also consider the implications on public health surveillance and disease control (168).

When dealing with rare or uncommon diseases; the number of affected individuals can be small, research may not be a clinical priority, clinical guidelines for management may not exist and patient forums may not have been established. In these circumstances, patient registries help us to understand the scale of the problem, establish prevalence (how many are affected), geographical location, demographics, and characteristics of disease. Over time the scope of a registry can evolve (a supportive mechanism for research funding and attracting health care providers). The purposes of rare disease registries can differ slightly and may also include: (1) Establishing connections between affected patients, families, and clinicians, (2) defining the natural history, evolution, risk, and outcomes of the disease, (3) to support

genetic research into the disease and (4) establishing a highly motivated and flexible patient base for developing new treatments, diagnostics, or screening techniques.

Disease registries work as a collaboration between researchers, consenting volunteer participants, and clinicians with a special interest in the disease in question. There are many potential benefits for all stakeholders involved.

Considering EUROPAC as a specific example; participants benefit from patient education throughout the registration process. This can involve realistic discussions between the individual and the clinician to help clarify that person's exact risk of developing cancer (more often than not, this risk is over perceived by the individual). There is also opportunity for the clinician to further educate participants on lifestyle adjustments, risk modifications and increasing awareness of alarm or red flag symptoms. These messages are often then passed on to the rest of the participant's family potentially resulting in other members of the kindred registering. Registries also encourage communication between members often resulting in the establishment of patient forums (this can be participant or registry led). In addition, once registered, many participants feel empowered with more credibility when discussing concerns or fears with other clinicians such as general practitioners for example. Registries may also be able to offer support for participants through patient/family networks.

For researchers, registries offer a detailed epidemiological data set with standardised data collection which can be interrogated using epidemiology study methods (observational) to test and refine assumptions on the natural history of the disease and other specified patient outcomes such as diabetes status, PDAC occurrence and the need for surgery. Registries facilitate the opportunity for healthy participants to donate vital biological research samples which may include blood, DNA, urine, and saliva for example. These samples, if collected prior to diagnosis of PDAC in this case, will prove invaluable for the future development of novel biomarkers in early disease.

For clinicians, maintaining patient registries are important as they can lead to improvements in patient care by providing data on which treatments work best in ‘real world’ situations. Registries can improve the chances of drug developments, better screening modalities and novel biomarker discovery. Working with industry.

There are disadvantages and caveats to be considered. Individuals with specific diseases (In this case FPC and HP) are very likely to have an inflated perception of their individual risk of developing PDAC and higher levels of anxiety (169). This anxiety can be amplified throughout all stages of the registration and recruitment process. However, perhaps the most potentially detrimental aspect is the inevitable inclusion of false positive families and inappropriate individuals onto these registries.

6.1.2 Pancreatic cancer

Pancreatic ductal adenocarcinoma (PDAC) is a highly aggressive disease and remains one of the most lethal cancers, with an extremely low 5-year survival (3-15%) that has remained unchanged in decades (170-172). The incidence of PDAC has been rising and it is predicted to be the second leading cause of cancer deaths by 2030 (173). Surgery with adjuvant chemotherapy remains the only potentially curative treatment. The ESPAC-1 trial established adjuvant chemotherapy as the standard of care in resected PDAC, ESPAC 4 demonstrated a significant improvement in 5-year survival (29%) with gemcitabine and capecitabine over the previous standard treatment with gemcitabine monotherapy of 16% (174), and more recently the PRODIGE 24 trial establishing the role of modified folfirinox in adjuvant therapy (175, 176) and ESPAC 5 confirming its role in the neoadjuvant setting (177). Despite these significant improvements in cancer treatment, prognosis remains poor as most patients with PDAC (80-90%) (178-182) (171-173) have locally advanced disease which limits curative treatment options, or distant metastatic

disease at diagnosis which precludes surgical resection, due to late presentation. The best survival can thus be achieved if tumours are early at the time of resection, but such tumours are rarely found as individuals are usually asymptomatic (183).

Symptoms caused by PDAC can be influenced by the location of the tumour within the pancreas. Tumours of the head of the pancreas classically cause obstructive jaundice. Tumours of the body and tail may cause vague and non-specific symptoms which can make detecting PDAC challenging.

The numerous factors that increase the risk of developing PDAC have been extensively reviewed and include age, tobacco consumption, alcohol excess, obesity, non-O blood groups, chronic pancreatitis, late onset diabetes mellitus, hereditary pancreatitis, cystic fibrosis, certain cancer family syndromes and a family history of PDAC. Genetic risks are responsible for 10% of all pancreatic cancers and are discussed later (184). PDAC is more likely to occur in older patients with 80% of pancreatic cancer arises in patients aged 60-80. Half of these patients are over the age of 70 and is unlikely to occur under 40 years of age (185). With an ever-aging population, the incidence and frequency of PDAC will increase. The lifetime risk of PDAC in the general population is around 1%. Individuals with at least 5–10-fold increased risk for PDAC are deemed individuals at risk (IAR) and are considered to be good candidates for screening.

Generic risk factors for PDAC within the general population are well described and have been summarised in table 13 below.

Table 13. Risk factors for PDAC in the general population (140) (186)

Risk factor for PDAC	Odds ratio (95% CI)
FH (≥ 1 affected with PDAC)	3.2 (1.8–5.6)
Excessive ETOH consumption (>85.5 u/week)	2.2 (0.9-5.6)
Tobacco smoking (>20/day)	2.0 (1.6-2.9)
High BMI ≥ 35	1.5 (0.9-2.5)
Diabetes Mellitus duration > 10 years	1.5 (1.01-2.2)

6.1.3 Hereditary pancreatitis – the risk of PDAC

To date, EUROPAC holds the largest series of individuals with HP in Europe. Landmark early work from the EUROPAC group gave the first accurate quantification of the risk of PDAC in individuals with HP (21). Howes et al demonstrated an increased cumulative pancreatic cancer risk with advancing age from 0.5% (95% confidence interval [CI]: 0.0 – 1.3%) at 40 years to 33.3% (95% CI: 19.0 - 47.5%) at 80 years of age (21). When considering cumulative risk from onset of symptoms, at 70 years from onset, the risk for cancer development was 44% (95% CI: 8.0 – 80%)(187).

PDAC can also vary between the different mutation variants. An individual with the highly penetrant R122H PRSS1 variant can have more than a 50-fold increased risk of pancreatic cancer, whereas those with the A16V variant often display a highly variable phenotype making PDAC risk in this cohort difficult to quantify. A patient with CFTR associated familial pancreatitis has a more modestly elevated risk (73), and those with sporadic chronic pancreatitis have a five-fold increased risk of pancreatic cancer (188), but this risk is too low to enable effective secondary screening in this group of patients.

The underlying genetic mechanism for increased PDAC risk in HP remains unclear. Some hypothesise that the malignant pre-disposition is completely independent of any genetic mutations and is purely related to the architectural changes seen within the pancreas from chronic pancreatitis related disease progression.

Overall, HP confers an approximate 40% lifetime risk of developing PDAC and therefore these individuals are a key target for PDAC screening research programmes. Due to their genetic predisposition to PDAC, EUROPAC offers registered individuals screening on a research basis. The screening protocol in HP is discussed in more detail later in this Chapter, and for FPC in Chapter 7.

6.1.4 Other risk factors for PDAC in HP

The link to PDAC in HP may be in part associated with smoking, (189, 190) and possibly diabetes mellitus (DM) (191, 192). Even in the absence of a history of hereditary pancreatitis, risk factors such as smoking, DM (Type 2) and chronic pancreatitis can account for up to a third of PDAC cases. Certainly, tobacco smoking and DM amongst others are risk factors for the development of PDAC in the absence of a history of hereditary pancreatitis. (193) In addition, smoking is a risk factor for the development of DM in the general population. (194)

6.1.5 Diabetes and PDAC risk

Diabetes mellitus is a group of diseases that are defined by persistent hyperglycaemia. (195) The most common form is Type 2 diabetes which is caused by an impaired insulin sensitivity and followed by an inadequate compensatory insulin response. Pancreatogenic diabetes (previously known as Type 3c diabetes mellitus) develops as a direct consequence of disease processes within the pancreas. It is characterised by a deficiency in the secretion of insulin and other hormones made by the β-cells and fibrosis of the islets of Langerhans (111, 196). In around three-quarters of cases pancreatogenic diabetes is caused by chronic pancreatitis with the remaining quarter of cases consisting of PDAC, haemochromatosis, cystic fibrosis and previous pancreatic resection (197). Pancreatic cancer causes β-cell dysfunction, β-cell death (apoptosis) and/or paraneoplastic sequalae leading to insulin resistance and hyperglycaemia and presenting as early DM (198).

The relationship between PDAC and DM is complex. Diabetes is both a risk factor and a symptom of early pancreatic cancer. Having diabetes does not cause a general increase in an individual's risk of developing cancer,(199) but it may increase the risk of developing particular types of cancer.(199, 200) Several studies have shown a higher prevalence of DM in individuals with PDAC compared to those with other cancer types or in control patients. (201-

203) Systematic reviews and meta-analyses have confirmed diabetes as a risk factor for PDAC reporting risk ratios between 1.8-2.2. (191, 204-206).

This risk is higher still in those with recent new onset diabetes (191, 207, 208) and in these patients' new onset DM may represent an early sign of PDAC. More recent studies have contradicted earlier beliefs that long standing diabetes mellitus did not confer an increased risk of PDAC, (209) and shown a long term diagnosis of diabetes mellitus roughly doubles your risk of developing PDAC (210). A systematic review identified an elevated risk of pancreatic cancer even in type 1 diabetes despite several decades between onset of the diabetes and the malignancy, (206) and type 2 diabetes mellitus can often precede pancreatic cancer diagnosis, sometimes by many years.(204, 211, 212)

Diabetes mellitus is both a risk factor and a symptom or consequence of early PDAC. Smoking and DM are common risk factors for PDAC. A synergistic relationship exists between smoking and diabetes in conferring cancer risk so the link between diabetes and pancreatic cancer could be via a common association with tobacco (213). DM as a manifestation of early PDAC offers a window of opportunity for early disease detection, screening, and intervention.

6.1.6 Screening for PDAC

Earlier detection of pancreatic cancer is paramount to improving prognosis, survival times and the proportion of patients who can be offered potentially curative surgery. On face value PDAC may sound like a good target for screening; earlier detection of PDAC including the identification and resection of precursor pancreatic lesions before the development of invasive malignancy should improve survival. However, a population-based screening program is not the answer. There are numerous challenges and the factors and processes associated with malignant transformation of precursor lesions of the pancreas are not fully understood notwithstanding.

The Wilson-Junger criteria emphasise the importance of consideration of costs, having adequate health care provision to deal with the extra work

created by screening, and having an accepted treatment for those with the disease.

Primary population-based screening for PDAC is currently not feasible due to insurmountable logistical and financial challenges which would make such a screening programme ethically unsound. Firstly, the diagnostic imaging modalities available to us as screening tests do not offer sufficient specificity to avoid false positives exceeding the number of true positives. Despite its high mortality rate PDAC thankfully has only a 1.3% lifetime risk (170) and is a low incidence disease (8-12 per 100,000 per year) and even for those over 75 years of age (the highest risk age group) it is less than 120 per 100^5 (214). Thus, a screened population of 10,000 participants may only yield one true positive case and a screening test with greater that 99.9% specificity would be required to avoid having more false positives than true positives.

Secondly, the only intervention that offers a potential cure is surgical resection and systemic chemotherapy (neoadjuvant or adjuvant). Surgery on the pancreas caries a relatively high risk of associated morbidity and mortality (215) and some individuals in the population may not have the physiological reserve to be deemed 'fit enough' for this surgery at pre-operative assessment. In this scenario, it would be unethical to screen these individuals in the first place.

This thesis has identified and discussed several patient subgroups who fall into the categories of having complex pancreatic disorders/diseases or are at increased risk of developing PDAC. These groups may provide an opportunity for the early detection of pancreatic cancer with the possibility of screening.

Positive predictive values can be improved by screening a population of high-risk individuals who have an increased incidence of PDAC (secondary screening). Several groups at higher risk of developing PDAC have been identified. Those with an inherited or familial risk (216, 217) (such as those included in the EUROPAC registry), individuals with certain cystic lesions of

the pancreas (218, 219), individuals of increasing age (>50 years), a smoking history, and most recently those with new onset diabetes (220).

Screening for pancreatic cancer may become feasible within a 'secondary screening' setting, but currently screening for PDAC is undertaken on a research only basis in populations with autosomal dominant predisposition for pancreatic cancer (221).

6.1.7 Screening consensus - The International Cancer of the Pancreas Screening consortium

6.1.7.1 Individuals at risk (IARs)

Consensus recommendations for secondary screening of high risk groups were proposed at the Fourth International Symposium on Inherited Diseases of the Pancreas in 2003 (222). There was agreement that secondary screening should only be carried out in patients with a strong genetic susceptibility including Hereditary pancreatitis (PRSS1) and Peutz-Jeghers' syndrome (STK11/LKB1 mutations). Subsequently the International Cancer of the Pancreas Screening Consortium (CAPS) was formed in 2010 with the specific objective of developing statements on screening in Individuals at Risk (IAR) with an inherited disposition to PDAC.

The definition of an IAR was expanded in the 2011 CAPS meeting and in a subsequent white paper to include individuals whose lifetime risk of developing pancreatic ductal adenocarcinoma is higher than 5%; FPC, PJS, FAMMM, HBOC, and HNPCC mutation carriers with >1 affected first-degree relatives, and those with mucinous cystic lesions of the pancreas(223, 224).

6.1.7.2 How should individuals be screened?

Recommendations from the 2011 CAPS meeting included only undertaking screening in IARs with a view to detecting relevant lesions in individuals fit enough and suitable for potential pancreatic resection. Potentially relevant screening findings were defined as early PDAC (T1 N0 M0 R0), grade 3 PanIN, and high-grade BD or MD IPMN *(223)*.

The CAPS consortium failed to reach consensus on the ages for initiating and stopping screening, on the imaging modality of choice (although EUS and MRI were recommended) by 75% of participants, or on screening intervals. Generally screening is Initiated around 40-50 years of age or at 10 years younger than the youngest PDAC diagnosis within that family.

In addition, screening should ideally be undertaken at a pancreatic tertiary centre as part of a recognised research programme.

There are established research screening programmes for PDAC in existence. They include North American National Familial Pancreatic Tumor Registry (225) The German National Case Collection for familial pancreatic cancer (226), and EUROPAC(227), which is discussed in detail below. Screening protocols and modalities vary between the programmes, but generally have baseline cross-sectional imaging with serum blood tests and tumour markers, followed by annual non radiating imaging including MRI and/or EUS.

6.1.8 The European Registry of Hereditary Pancreatitis and Familial Pancreatic Cancer

EUROPAC is a collaboration of clinicians and scientists, established in 1997 and is now administered from Liverpool on a hub and spoke basis around the United Kingdom and collaborators in other European Countries. EUROPAC recruits families with either Hereditary Pancreatitis (HP) or Familial Pancreatic Cancer (FPC) who both have an increased risk of developing pancreatic cancer and offers secondary screening on a research basis.

6.1.8.1 EUROPAC history

The phenomenon of FPC was first described as part of a case series by Lynch et al (228-232). following on from this publication, the National Familial Pancreas Tumour Registry (NFPTR) was the first pancreatic disease registry in the world established at the Johns Hopkins by Ralph Hruban in 1994. Groups in Europe closely followed such as the EUROPAC at Liverpool

University established in 1997, and FaPaCa at Phillips University (Marburg, Germany) established in 1999.

From its conception, EUROPAC focussed on identifying individuals at high risk of pancreatic cancer. Including recording patient demographics, symptomatology, and genetic data in individuals with hereditary pancreatitis (HP) and familial pancreatic cancer (FPC).

In some of the EUROPAC FPC families a known causative mutation has been identified (mutations in *BRCA2*, *CDKN2a, STK11* or mismatch repair genes). These are associated with autosomal dominant predisposition that can be confirmed by segregation of the mutation with cancer cases. However, in most of our families DNA sequencing has not been possible in enough cases to confirm segregation, so we cannot rule out low penetrance in some of our families despite the presence of known causative mutations in screened individuals (e.g. a BRCA2 mutation cannot be guaranteed to equate to very high risk because the individual may have a protective genetic background not seen in high-risk families with BRCA2). Historically, EUROPAC has been unable to distinguish families with genuine autosomal dominant predisposition from families where the cancer cluster occurs by chance or due to a polygenic predisposition. However, with the development on Family index (FI) to Family risk (FR) we are now able to calculate FR from information derived from the database and family pedigree. This further risk stratification of participants has helped refine the population who are at the highest risk of PDAC and thus allowed focus of resources and intensity of screening.

Regarding the hereditary pancreatitis arm, the subsequent discovery of the causative mutation for HP (PRSS1) and identification of other polygenic mutations such as SPINK-1 and CFTR as risk factors for idiopathic pancreatitis, facilitated a rapid increase in registrants. Results from the EUROPAC database were published in several landmark papers: The Liverpool group were the first to accurately quantify the risk of pancreatic cancer in Hereditary Pancreatitis (21), the first to accurately determine penetrance for the different types of HP mutation (22), and also showed that

SPINK1 trypsin inhibitor gene variant (N34S) acts to lower the threshold of developing pancreatitis (233). Data acquired through the EUROPAC database was also used in a collaborative study that showed that CFTR mutations give a four-fold increased risk of (idiopathic) chronic pancreatitis (234).

EUROPAC opened its secondary screening study arm in 2007 and launched a tri-annual screening protocol for consenting individuals from affected HP and FPC kindreds due to the recognised increased risk of pancreatic cancer. Pre-2007, affected individuals on the HP registry were offered screening on clinical grounds due to the 40% lifetime risk of PDAC (21).

There are currently numerous established International and National registries of FPC families and other high-risk groups in the USA, Europe, Canada, and Australia, and now newly established in Japan. Many of these registries are associated with screening programs or pilot studies. These are reviewed subsequently.

6.2 Aims and objectives

The EUROPAC study was established with the initial aim of identifying those individuals at higher risk of developing PDAC. Here we present the screening outcomes for HP kindreds in EUROPAC and the development of further risk stratification tools to aid and improve future screening.

Using epidemiological and demographic data from these HP families within the EUROPAC cohort, this study aimed to describe the relationship between smoking, diabetes, and pancreatic cancer risk in a well phenotyped population know to have hereditary pancreatitis. Diabetes is both a risk factor and a symptom or consequence of early PDAC. HP is associated with diabetes and cancer so represents an ideal population to study the interaction of the two.

6.3 Methods

The remaining sections of this chapter will discuss the HP arm of the EUROPAC database, registry, and screening program. The FPC arm is covered in chapter 7.

6.3.1 Patients and ethics

The European Registry of Hereditary Pancreatitis and Familial Pancreatic Cancer (EUROPAC) was established with local ethical committee approval in 1997(235) and subsequently rationalised in 2007 for HP (MREC 04/0/010).

EUROPAC has ethics approval (MREC 03/8/069) to recruit families with an increased risk of pancreatic cancer. EUROPAC screening, initially performed under local ethical committee approvals, was rationalised in 2007 for both FPC (REC reference 07/H1211/96) and HP (REC reference 07/H1008/153). Referrals for registration for both HP and FPC kindreds were accepted via clinicians, clinical genetics service, or from private individuals who had identified their own risk.

All relevant clinical information for cases referred to EUROPAC were individually reviewed by the multidisciplinary EUROPAC committee prior to acceptance on to the register. Following acceptance, written informed consent was obtained from both affected individuals and their referring clinicians prior to each completing detailed questionnaires.

Recruitment of HP families was from 22 countries. Figure 10 Demonstrates the geographical distribution of EUROPAC registered HP families.

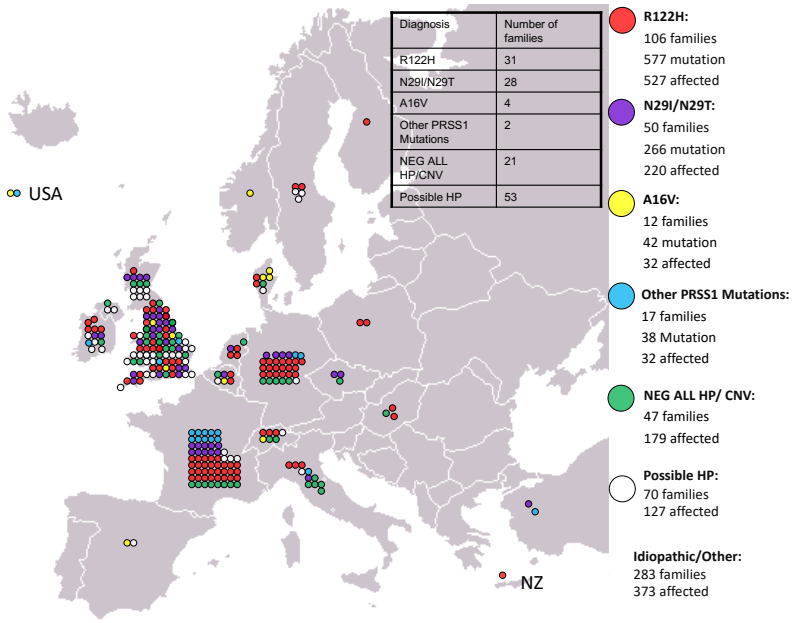


Figure 10. Geographical representation of the global extent of recruitment to EUROPAC

Secondary screening for pancreatic cancer on a research basis was offered to all individuals with HP over the age of 40 or less than 10 years younger than the youngest PDAC case in the family. To be eligible, participants had to have a diagnosis of HP confirmed, either with a proven PRSS1 mutation or a family history consistent with autosomal dominant inheritance. All eligible individuals were provided with information regarding participation in the secondary screening study for the detection of PDAC. However, recruitment for screening was patient led from this point, with approximately 40% uptake.

6.3.2 HP eligibility and inclusion criteria

6.3.2.1 Inclusion criteria for the EUROPAC HP registry

During my tenure as the EUROPAC Clinical Fellow the eligibility criteria for inclusion in the EUROPAC HP registry for HP kindreds included the presence of 2 first-degree relatives or 3 or more second-degree relatives, in 2 or more generations with recurrent acute pancreatitis and/or chronic pancreatitis in single kindred, for which there were no precipitating factors.

6.3.2.2 Inclusion criteria for the EUROPAC secondary screening program for HP

Any member of a hereditary pancreatitis family who has been confirmed to carry a causative PRSS1 mutation.

An affected member of a family consistent with HP who has tested negative for known causative PRSS1 mutations.

6.3.2.3 Exclusion criteria

- Any participant who is incapable of providing informed consent.
- **For genetic testing:** Any individual who does not consent to be informed of clinically significant results. Genetic testing for a predisposition for pancreatitis will still be carried out on individuals who have expressed a wish not to be informed following detailed discussions on the limitations of a right not to know in this case; testing will be carried out only if individuals wish to have testing just for research.
- **For screening:** Individuals of less than 40 years of age or 10 years younger than the youngest case in the family will be excluded.

6.3.2.4 Withdrawal criteria

- Requested by the registered individual (only). No explanation or reason is required.
- Identified contraindication to any of the screening modalities used, for example unable to tolerate endoscopy for EUS.
- Loss of competency and or capacity to give continued consent.
- Pregnancy

Identifiable data or tissue already collected with consent would be retained and used in the study. No further data or tissue would be collected, or any other research procedures carried out on or in relation to the participant.

6.3.3 The classification and definition of HP

The classifications or subtypes of hereditary pancreatitis used for subgrouping of kindreds and individuals within the EUROPAC registry and in work as part of this thesis are as follows:

6.3.3.1 True HP

Individuals from kindred carrying a confirmed mutation of the cationic trypsinogen gene *PRSS1* and/or a pattern of pancreatitis occurrence within the kindred that would be fully consistent with an autosomal dominant inheritance pattern. Kindreds were then classified and recorded on the database under the *PRSS1* mutation they were confirmed to carry (p.R122H, p.N29I or p.A16V).

6.3.3.2 Negative for all known mutations

A 'Neg All' (negative for all known mutations) classification was given if there was a family history consistent with an autosomal dominant pattern of pancreatitis but with the absence of a proven *PRSS1* mutation (wild type genotype for *PRSS1*) among affected individuals. Such individuals continue to be included as the EUROPAC scientific committee maintains the presumption that there are further, yet unidentified mutations, which may act in a similar way to *PRSS1* mutations.

6.3.3.3 Copy number variation

Copy number variation (CNV) is a type of structural variation, usually a type of duplication or less commonly a deletion event that affects a whole gene or sections of a gene. The area affected may then become more active. There has been reported CNV associated with both duplication and triplication of a 605kb segment on chromosome 7q35 associated with chronic pancreatitis. This has led to both increased copy number of PRSS1 as well as PRSS2.(236)

6.3.3.4 Familial Idiopathic pancreatitis

Familial idiopathic pancreatitis cases include a cluster of symptomatic individuals within a kindred who have a non-autosomal dominant inheritance

pattern. In addition, sporadic causes, Copy Number Variant (CNV), PRSS1 or CFTR mutations should be excluded.

6.3.3.5 Affected individuals

Symptomatic individuals within a kindred.

6.3.4 Data collection

6.3.4.1 Patient health questionnaires

The EUROPAC HP registry functioned(s) largely as an observational study. Information and data are acquired in a voluntary basis from consenting, eligible and registered HP kindreds via the use of ethically approved personal and family history questionnaires. The 'EUROPAC Study Questionnaire of Hereditary Pancreatitis (HP)' is made up of several sections including epidemiological data, personal data, demographics, exposure to known risk factors, smoking status (Current, ex or never and quantitative data), alcohol intake status, dietary factors, medical history (including diabetes status, pancreatitis, and other intra-abdominal conditions), age of symptom onset and finally a surgical history section. This process is supported by clinical consultations. Data are entered onto a secure database (Progeny version 8.01). Data were stored in a pseudo anonymised fashion and in accordance with the UK Data Protection Act (1998) and matched, wherever possible, with DNA samples kept under the care of the Merseyside and Cheshire Genetics Service.

Confirmation of pancreatic cancer diagnoses or other significant diagnoses were made either via histology reports, local and national cancer registries or by obtaining a copy of the death certificate.

6.3.4.2 Smoking status

Smoking status was classified as either; **Current:** Smoking at the time of diagnosis with diabetes, **Non:** Not smoking prior to diagnosis with diabetes, and **Ex:** Smoking before onset of diabetes and giving up also before diagnosis of diabetes).

6.3.5 EUROPAC screening protocol in HP

This section describes the HP EUROPAC screening protocol that was followed during my tenure as the EUROPAC Clinical fellow until Oct 2016. The protocol that is currently being employed has evolved from this and is summarised in the discussion.

Following the appropriate informed consent and registration with EUROPAC, Participants with HP underwent baseline imaging (pancreas protocol computed tomography and a one-off endoscopic ultrasound of the pancreas) in addition to serum fasting glucose, HBA1c and Ca-19.9 levels. Collaboration within the EUROPAC study group has allowed the opening of several screening sites across the UK and Europe. The UK based EUROPAC screening centres and number of participants screened for both FPC and HP are shown in Figure 11.

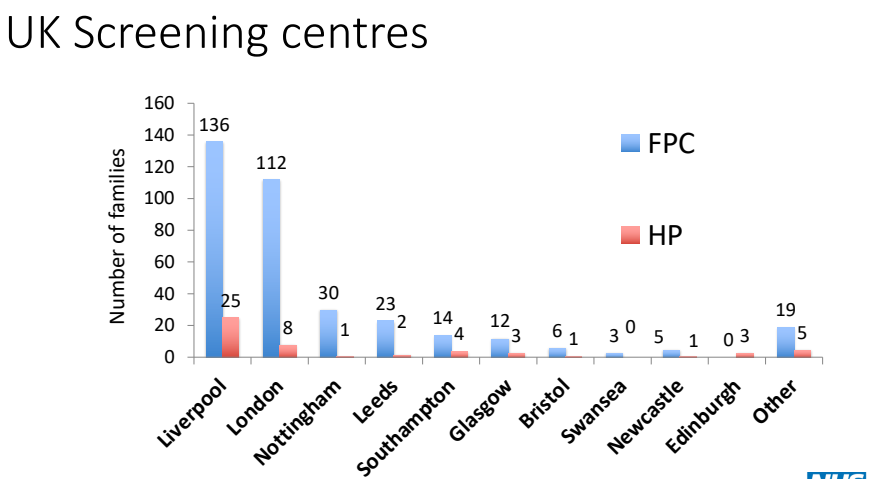


Figure 11. The UK based EUROPAC screening centres and number of participants undergoing screening as of October 2016

The screening protocol for HP and FPC participants are illustrated in Figure 12. The protocols differ between these two groups due to concerns over the ability of EUS to detect small pancreatic lesions in an already fibrotic and diseased pancreas as in often seen in those with HP. Both EUS and CT have

sensitivities greater than 90% for diagnosis pancreatic lesions (237, 238), and EUS has been reported as having specificity as high as 98% in the 'normal' pancreas (239). This specificity falls to around 64% in the presence of pancreatitis. Therefore, within EUROPAC screening, EUS is the imaging modality of choice in FPC kindreds who are expected to have 'normal' pancreata, whereas CT is the follow-up imaging modality of choice in those with HP.

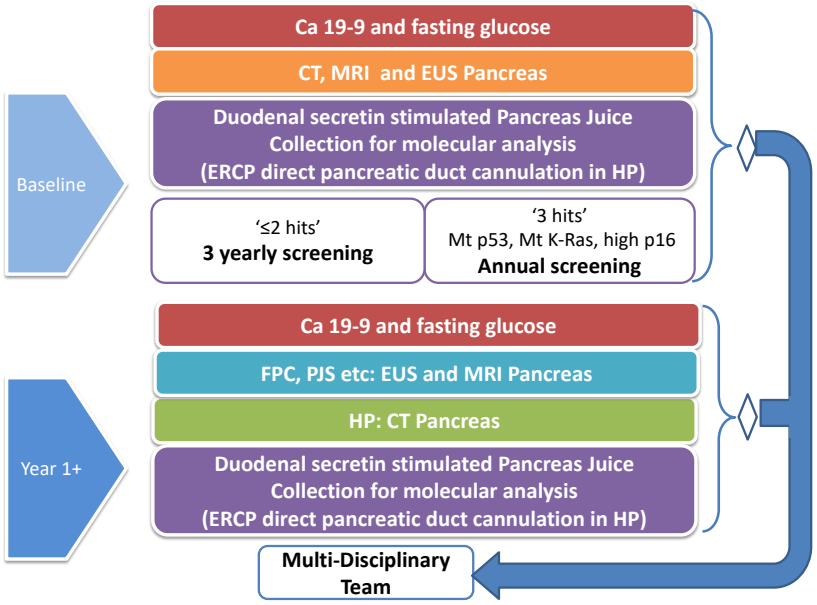


Figure 12, Comparison of screening protocol for HP and FPC as of Oct 2016

Consenting participants are also offered ERCP and molecular analysis of pancreatic juice, with secretin stimulation. If no cancer associated genetic abnormalities are identified, participants entered a three yearly screening cycle, with repeat CT and ERCP with pancreatic juice collection staggered over this period. For those who did not consent to ERCP, or those with an identified cancer associated mutation in their pancreatic juice, there was an annual pathway consisting of repeat blood testing and CT. All abnormalities (including cancer associated mutation in pancreatic juice) were discussed at the supra-regional pancreatic multi-disciplinary team meeting.

6.3.5.1 ERCP and molecular analysis of pancreatic juice

From 2003, individuals with HP were also offered the opportunity to have molecular analysis of their pancreatic juice via ERCP collection following intravenous Secretin® (Sanochemia, Germany) (1iu/Kg) stimulation. A maximum of 10mls was aspirated directly from the cannulated pancreatic duct. The juice was assessed for the presence of three cancer associated molecular markers: K-RAS2 mutations, CDKN2A methylation and TP53 mutations. Consenting individuals had a day-case procedure ERCP performed by a consultant endoscopist (member of the EUROPAC study group) under sedation.

ERCP and pancreatic juice collection in FPC kindreds was abandoned in 2008 as the EUROPAC study group observed an unacceptably high rate of post ERCP pancreatitis (7 out of 16 participants = 43.8%). A trial of post-ERCP pancreatitis (PEP) prophylaxis in FPC (self-expelling plastic stent placement in the main pancreatic duct (MPD) and 50mg per-rectum diclofenac) was conducted in 2009. The study showed a significant reduction in the number of FPC individuals developing post ERCP pancreatitis post juice collection (43.8% to 9.7% [p=0.020]) however, the EUROPAC study group felt a rate of nearly 10% was still too high to justify its inclusion in FPC screening (240).

There were no cases of PEP in the HP kindreds. The low risk is attributed to abnormal ductal anatomy seen in CP and MPD dilatation. Molecular analysis of pancreatic juice remains a useful addition to risk stratification in this group as reliance on traditional imaging methods can be challenging in an already diseased pancreas.

6.3.6 Statistical analysis

Continuous variables and data are presented as median with inter-quartile intervals (IQR) unless data are clearly non-parametric in which case data will be presented as means and standard errors of means. Categorical data are displayed as tables of counts and associated percentages. Nominal data will

be compared using Pearson Chi Square or Fisher's exact testing as appropriate.

Associations of factors across patient groups were carried out using a two tailed Mann-Whitney U test for continuous data and Fisher's exact test for categorical variables. Risks are presented as odds ratio with associated 95% confidence intervals and are obtained from the parameters of univariate logistic regression models. All analyses were carried out using 'R' statistical software. P values are assessed at the 0.05 level throughout."

Multivariate modelling was not attempted due to the small number of events.

Follow up data were analysed using the Schempers reverse Kaplan Meier method. Follow up was calculated in years as presented as either; 'person follow up years' which were calculated from birth and is the most conservative measure, or 'screening follow up years' which were calculated from the initiation of the first screening event/cycle. Clinically relevant lesions (CRL) were defined as a pancreatic finding that may affect prognosis and/or survival and/or would require and intervention or treatment as standard practice.

6.3.6.1 Survival analysis

Survival analysis was performed by regression or the method of Kaplan and Meier. This was performed for age of onset of pancreatitis, diabetes, malabsorption, and cancer etc. The level of significance in these data was tested using log rank with a two-sided 5% significance level. The R package 'survival'; 'cmprsk'; and 'crrSC' for Kaplan-Meier curves and Cox proportional hazards modelling will be used for cause specific survival analysis.

6.3.6.2 Censorship

Censorship was performed to allow for competing risks. Pancreatic resection alters an individual's subsequent risk of developing PDAC and therefore patients were censored if they underwent was a pancreatic resection prior to development of diabetes (at the age of resection).

Survival analyses of diabetes, malabsorption and cancer included censorship at the time of surgery if they underwent a resection before event time. Sadly, the majority of patients die withing months of a diagnosis of PDAC. Date of death was easier to verify and thus death was taken as the main outcome measure.

6.3.6.3 Regression models

The EUROPAC study group preferred to use the Cox proportional hazards model for regression analysis as it can be extended to address complications in data such as frailties due to genetic similarities among family members (leading correlated survival times). Standard Cox model assumes independence across observations, which is obviously not the case when multiple observations are taken from individuals in the same family.

Time varying covariates such as the age of diagnosis of diabetes were used in developing the regression model rather than just binary factors (e.g. diabetes yes, no).

The following formula was used to model univariate analysis when investigating relationships between pancreatitis and diabetes:

$$\lambda_k(t; X \mid Z_k) = Z_k \lambda_0(t) e^{\beta x_{ik}(t)}$$

λk (t) is the hazard function for the kth proband.
i represents each individual within the family.
xik denotes the value of the covariate (time-varying in the case of age of diagnosis of secondary diseases) of the ith member from the kth proband. Zi are unobserved cluster-specific random effects (frailties), which may be due to shared environmental exposure or common genotype.

It was assumed that Zi were independently and identically distributed random variables, which for convenience we have assumed to follow a gamma distribution with mean 1 and variance θ(241). In multivariate analysis, correlation between pancreatic resection and the subsequent risk of pancreatic

cancer was also taken into account using the model $\lambda_k(t; X \mid Z_k) = Z^{\alpha}{}_k \lambda_0(t) e^{\beta x_{ik}(t)}$ (242).

A sensitivity analysis was performed to test whether diabetes was a symptom or predisposing factor for cancer. In the main analysis the assumption was that diabetes was predisposing, while in the sensitivity analyses diabetes was assumed to be a symptom of cancer if it was diagnosed within x years prior to diagnosis of cancer (x = 1, 2, 3, 4, 5 or 10 in 6 separate analyses). If cancer was diagnosed ≤ x years after diagnosis of diabetes, date of cancer diagnosis was brought forward to date of diagnosis of diabetes and diabetes status was considered negative (as the diabetes is assumed to be due to cancer). If cancer was diagnosed > x years after diagnosis of diabetes, data were unchanged. If a patient had < x years of follow up from date of diagnosis of diabetes but did not develop cancer, dates of diabetes and cancer were both censored at date of diagnosis of diabetes. If a patient had ≥ x years of follow up from the date of diagnosis of diabetes, data were unchanged.

6.4 Results

6.4.1 HP registry

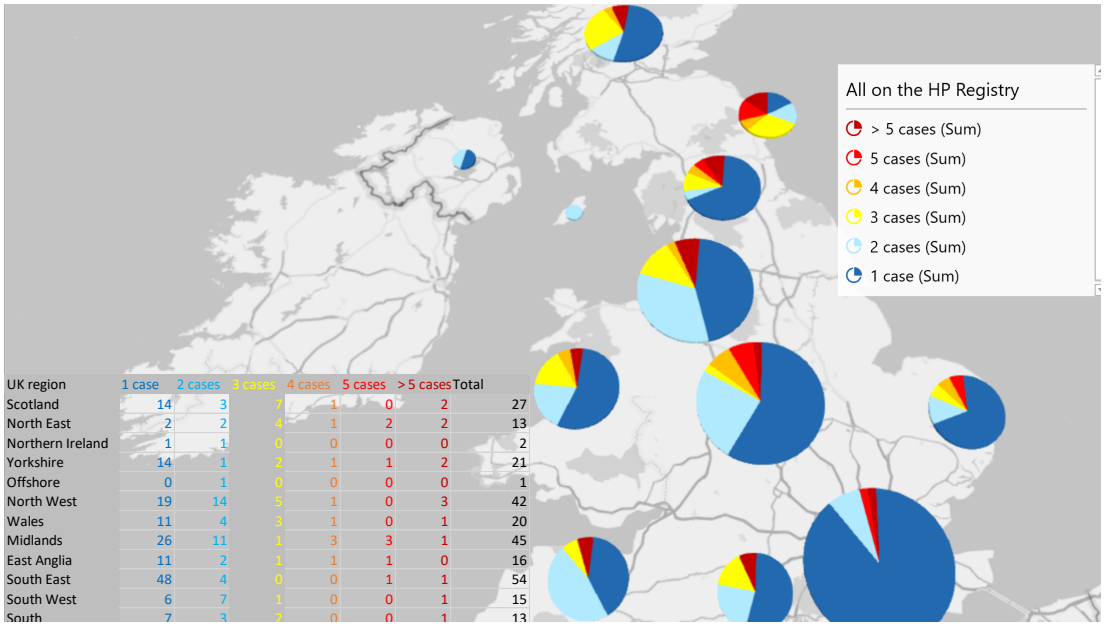
Individuals with R122H, N29I and “Neg All” mutations who had been entered on to the EUROPAC HP registry between 1999 and Oct 2016 were included. 8,831 individuals from 577 families were included on the EUROPAC registry. Data were available and analysed on 1008 individuals from 199 families with confirmed or assumed disease mutations (872 affected individuals) who fitted the criteria for HP. There were 47 cases of confirmed cases of pancreatic cancer occurring in affected individuals on the registry and 3 cases in unaffected individuals. A description of families and affected individuals is given in Table 15.

Table 14. Summary of HP database

Country	p.R122H families	p.N29I families	A16V families	NEG ALL HP families	Copy number variance	Other mutation	Familial Idiopathic Pancreatitis (FIP)	Possible HP	CFTR families	SPORADIC	TOTAL
UK	30	26	3	29	1	3	28	47	16	139	322
France	29	10	1	3	5	10	6	3	0	1	68
Germany	19	4	0	5	0	1	0	1	0	3	33
Eire	5	2	0	1	0	0	2	4	1	12	27
Denmark	2	0	3	1	0	0	4	1	1	12	24
Belgium	2	1	1	1	0	0	2	1	0	6	14
Italy	3	1	0	4	1	1	2	1	1	0	14
Netherlands	2	1	0	1	0	0	1	0	0	0	5
Finland	1	0	0	0	0	0	1	0	0	1	3
Greece	0	0	0	0	0	0	0	0	0	1	1
Sweden	2	0	0	0	0	0	0	3	0	8	13
Switzerland	3	0	1	2	0	0	0	1	0	1	8
Hungary	2	0	0	1	0	0	0	0	0	4	7
Czech	0	2	0	1	0	0	3	0	0	0	6
Spain	0	0	0	0	0	0	0	1	1	1	3
Turkey	0	1	0	0	0	1	1	0	0	0	3
Norway	0	0	1	0	0	0	0	0	0	0	1
Poland	2	0	0	0	0	0	0	0	0	0	2
Portugal	0	0	0	0	0	0	0	0	0	1	1
Sri Lanka	0	0	0	0	0	0	0	0	0	1	1
USA	0	0	1	0	0	1	0	0	0	0	2
TOTAL	102	48	11	49	7	17	50	63	20	191	558

Table 15. Relative frequencies of HP kindreds by type in the EUROPAC database

Mutation	Families (N)	Affected individuals	PDAC cases
p.R122H	101	477	25
p.N29I/T	48	198	11
p.A16V	14	30	3
Other PRSS1	15	27	1
Neg All HP/CNV	49	181	18
Possible HP	64	109	3
Familial IP	67	126	27
CFTR	22	32	1
Sporadic	196	211	3
Total	576	1391	92



UK region	1 case	2 cases	3 cases	4 cases	5 cases	> 5 cases	Total
Scotland	14	3	7	1	0	2	27
North East	2	2	4	1	2	2	13
Northern Ireland	1	1	0	0	0	0	2
Yorkshire	14	1	2	1	1	2	21
Offshore	0	1	0	0	0	0	1
North West	19	14	5	1	0	3	42
Wales	11	4	3	1	0	1	20
Midlands	26	11	1	3	3	1	45
East Anglia	11	2	1	1	1	0	16
South East	48	4	0	0	1	1	54
South West	6	7	1	0	0	1	15
South	7	3	2	0	0	1	13

6.4.2 Secondary screening for PDAC in HP: results of prospective screening 1999-2016

Here we report the outcomes of a prospective study of 37 patients with HP who underwent screening for pancreatic cancer between 1999–2016). These 37 participants came from 22 separate HP kindreds (R122H=11, N29I=7, no mutations=4).

Participants were followed up for a median of 52.9 (IQR 12.88-112.7) months, with a median of 4 (IQR 2-6) investigations being performed each.

Mutation Type	Number of Kindreds	Number of individuals	Participants
R122H	11	20	
N29I	7	13	
"Neg all"	4	4	
Total	22		37

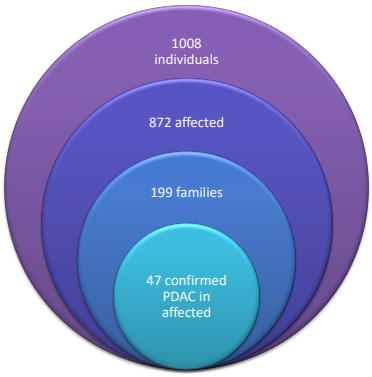


Figure 13. Diagrammatical summary of EUROPAC HP registry (right) and HP screening participants in the UK cohort (left) 1999-2016

6.4.2.1 Clinically relevant lesions

Clinically relevant lesions (CRL's) identified during EUROPAC HP screening.

- One MD-IPMN (subsequent to screening developed (and died of) metastatic breast cancer).
- Two PDAC.
- Three pancreatic resections: Low grade PanINs (1a/b and 2)
    - Of which, two resections were performed for abnormalities on molecular analysis.

Participants were followed up for a total of 2062 'person' follow-up years or 169 screening follow-up years. A clinically relevant lesion (CRL) was identified in 13% of participants (5/37). The CRLs were all identified within a maximum of 12 follow-up years. The screening yield was 1 CRL per 412 person follow-up years / 1 CRL per 34 screening years follow-up). The cumulative incidence of clinically relevant lesions (CRLs) in the study period was 11.3% and 15.3% at 2 years and 5 years respectively.

6.4.2.2 ERCP and PJMA

A total of 27 ERCP procedures were undertaken in 15 (1 missing result) HP individuals as part of EUROPAC secondary screening. There were no reported cases of post ERCP pancreatitis in this group. Two patients had abnormal PMJA. One had mtK-ras (p.G12V), mtTP53 (p.G245D), and CDK2NA promoter methylation >50%. The other had mtK-ras (p.G12R), wtTP53, and CDK2NA promoter methylation =17.6%. Both proceeded to pancreatic surgery as a result following review at both the EUROPAC study group meeting and the supra-regional pancreatic multidisciplinary team meeting.

6.4.2.3 Pancreatic resections during screening

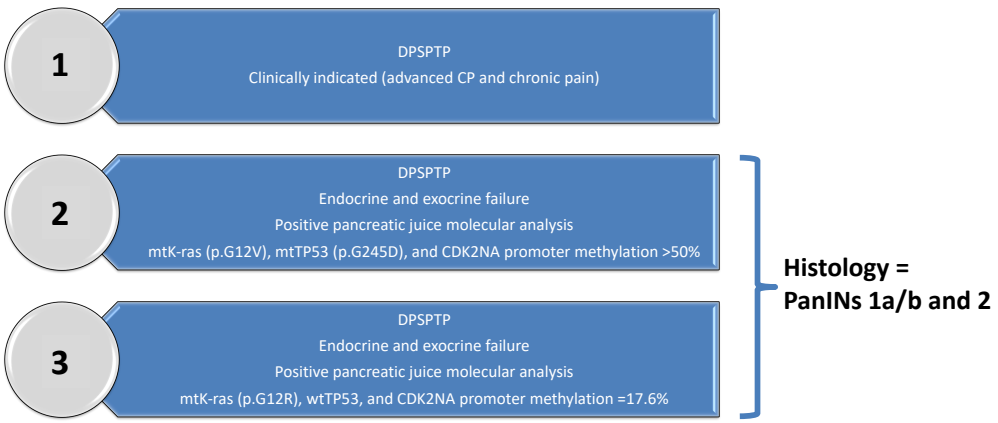


Figure 14. Histopathological analysis of pancreatic resections performed in HP patients during EUROPAC HP screening.

Three underwent total pancreatectomy: one clinically indicated (advanced chronic pancreatitis). The remaining two patients (both with endocrine and exocrine failure) had positive PJMA. Both cases had Low grade PanIN (grades 1A/1B, and 2) on histological examination.

6.4.2.4 All-cause mortality

There were 6 deaths from all causes: 2 PDAC, 2 extra-pancreatic malignancies, 1 cardiac failure, one unknown.

6.4.2.5 Protocol deviations

Fifteen participants (40.5%) had protocol deviations, two of whom developed inoperable PDAC. These were the only two participants to develop pancreatic cancer. One withdrew consent for inclusion in the screening programme due to a history of severe agoraphobia and was unable to attend hospital for screening investigations. They were aware of their risk of developing PDAC at the time of withdrawal and had capacity. The other missed having a baseline CT scan performed, where it was decided against advice and against the protocol, that an EUS would be performed instead. This failed to differentiate changes of CP with a cancer. PDAC was identified in the following screening cycle. At this point it was advanced/ unresectable. In summary the protocol deviations included: incorrect baseline investigations being performed (e.g. CT being missed), and/or inappropriate Follow up screening imaging being requested (E.g. EUS instead of CT) and/or participant not attending follow up appointments and therefore screening not being requested. Protocol deviation may also have been heavily influenced by level of participant engagement in the study. It is worth noting that out of the 15 patients with protocol deviations, twelve withdrew from annual screening. Of note, a proportion of these patients have subsequently recontacted the study to re-enrol.

6.4.3 Refining PDAC screening in HP through risk stratification

6.4.3.1 Pancreatic cancer, smoking and diabetes mellitus

Forty-seven individuals affected by pancreatitis and three unaffected family members developed pancreatic cancer. This was confirmed histologically in 17 cases and by review of medical notes in the remainder. Lifetime risk upto 75 years for pancreatic cancer was 29.8% (95% CI: 20.8, 41.4) and 34.2% (95% CI: 23.1, 48.5) to 80 years. Cancer cases were seen in 24 individuals with p.R122H mutations, 12 with p.N29I mutations, one with p.A16V and 13 from ?Neg all families. The cumulative incidence of cancer was not associated with mutation status, mode of transmission or nationality. The age of death from pancreatic cancer did not correlate with gender or age of onset of

pancreatitis. An increased risk of cancer was seen in current smokers by univariate analysis (HR = 4.17 (95% CI: 1.49, 11.66); *P* = 0.0064) but not alcohol consumption.

6.4.3.2 PDAC and endocrine failure

Of the 47 cancer patients with pancreatitis, 26 had confirmed diabetes mellitus, 10 were confirmed not to have had diabetes and diabetes status was unknown for a further 11. Of those with confirmed DM who went on to develop PDAC, only 24 had a recorded age of diabetes diagnosis. Of these 24, nine developed diabetes within 10 years of developing cancer, six of these 10 patients died of pancreatic cancer within a year of developing diabetes. The remaining 15 cancer patients with diabetes died of cancer at a median of 21 (range: 10-42) years after diagnosis of diabetes mellitus. An increased risk of cancer was seen in patients who developed diabetes by univariate analysis (HR = 3.10 (95% CI: 1.28, 7.52); P = 0.0120).

6.4.3.3 Extended Cox proportional hazard model

This included 376 patients, 117 of whom had diabetes before last follow-up (16 of whom had developed pancreatic cancer) and 259 without diabetes (seven with cancer). Data were omitted on 238 participants; 167 of these had no reliable data DM status (9 with cancer); 62 of the omitted participants had diabetes but with no known age of diagnosis (3 with cancer). Nine patients were lost to follow up (one had PDAC), two with diabetes and seven without.

6.4.3.4 Smoking and diabetes are associated with an increased risk of PDAC

We confirmed that both smoking and the diagnosis of diabetes was associated with an increased risk of developing PDAC in our HP cohort. These relationships are demonstrated in Figures 15 and 16 respectively.

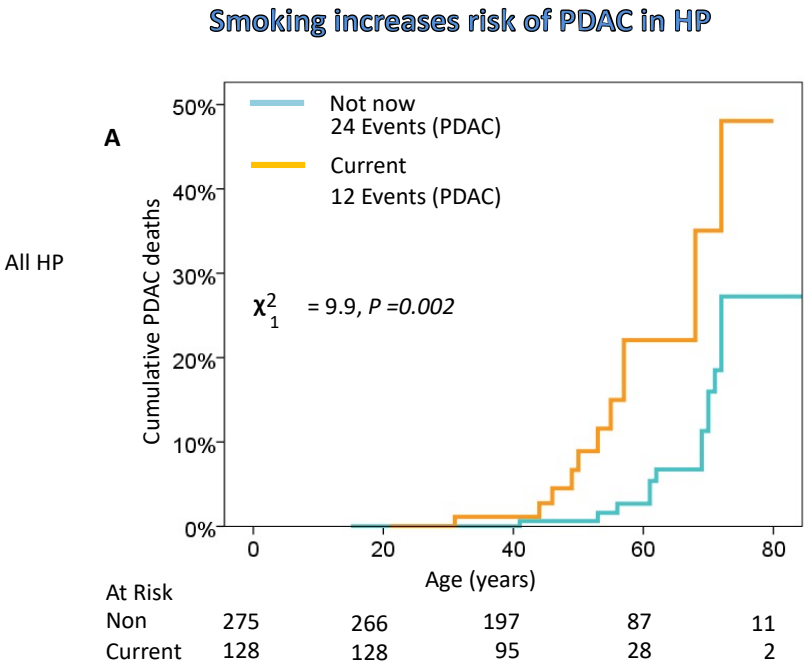


Figure 15. Individuals with HP that are current smokers have a higher risk of developing PDAC than non-smokers p=0.002.

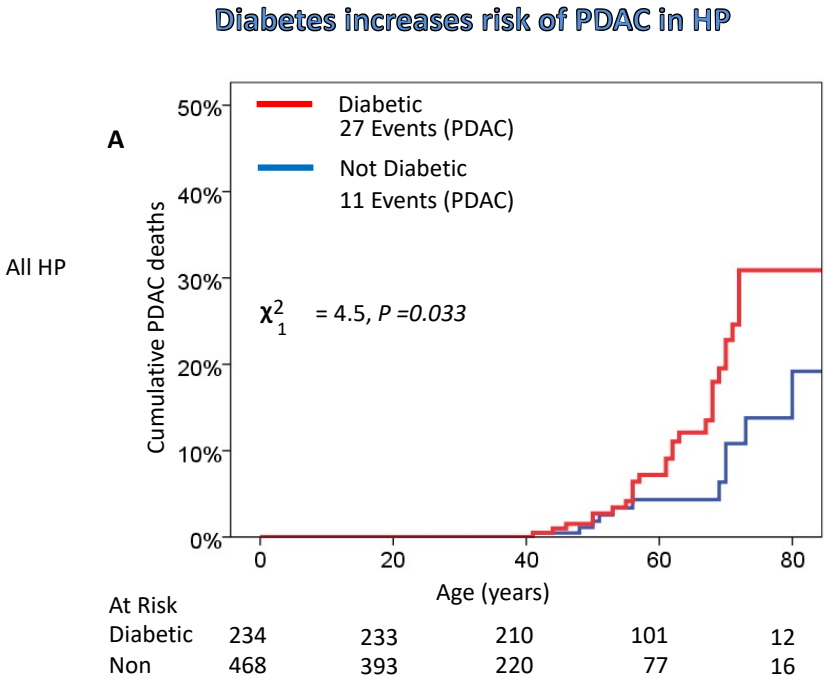


Figure 16. Individuals with HP that are diagnosed with diabetes have a higher risk of developing PDAC than non-diabetics p=0.033.

6.4.3.5 Smoking increases the risk of developing diabetes in HP

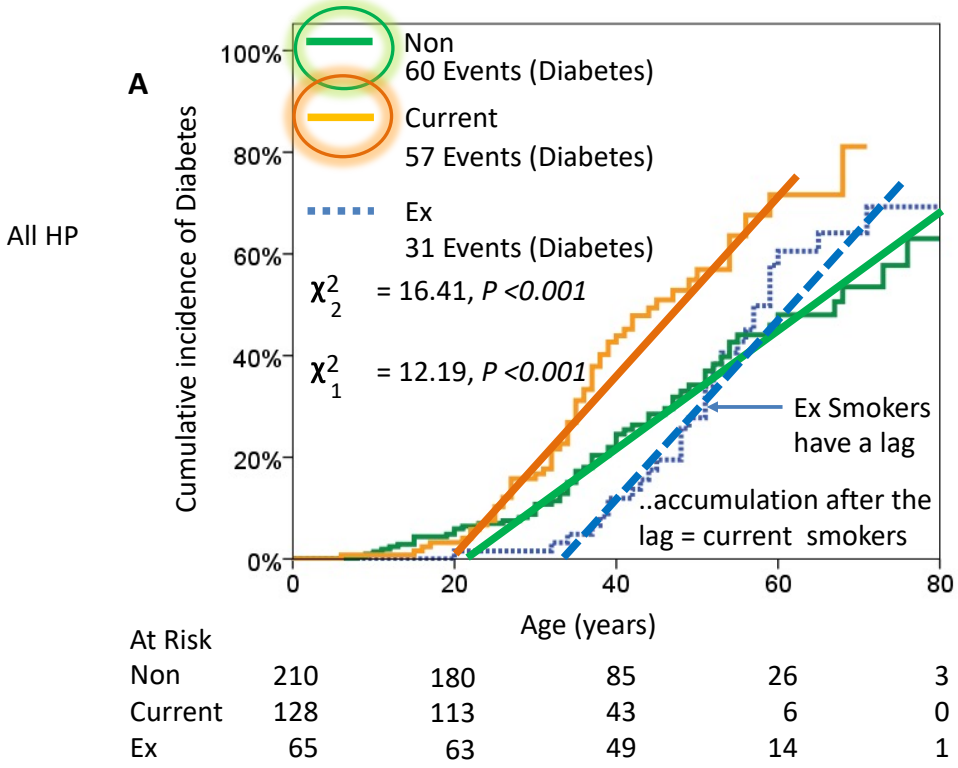


Figure 17. Smoking increases risk of developing DM in HP.

Current smokers developed diabetes earlier than non-smokers (X21 = 12.19, p< 0.001). Ex-Smokers developed diabetes later, but risk was consistent with that of current smokers (all mutation groups).

As shown in Figure 17, current smokers develop diabetes earlier than non-smokers. The Ex-smokers developed diabetes later as a consequence of a lag in development However, the rate of accumulation of diabetes after this lag in development seems more consistent with smokers than non-smokers (lag is expected as ex-smokers must start and stop smoking before the age at which they are diagnosed with diabetes). Each of the major mutation groups shows the same trend, although only reaching significance for the p.R122H group because of numbers included (Figure 18).

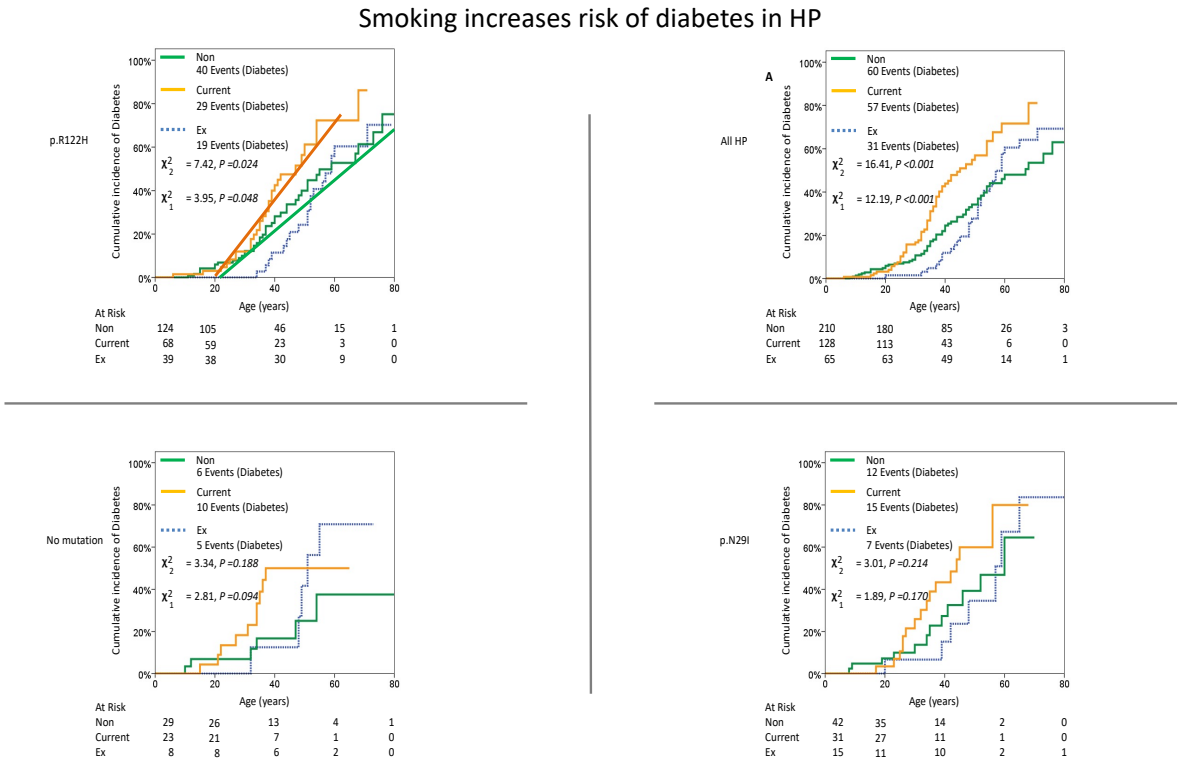


Figure 18. Smokers develop diabetes earlier than non-smokers.

A) All HP patients including the rare mutation groups (e.g. p.A16V). B) Only p.R122H. C) Only p.N29I D) Only NEG ALL HP. (Correlation performed by linear regression).

6.4.3.6 The temporal relationship between smoking and the diagnosis of Diabetes mellitus

There is no temporal relationship between smoking and diagnosis of diabetes. If smoking increases the risk of developing diabetes for all HP patients, then a linear regression of a plot of age of starting to smoke against age of diabetes should give a positive gradient. For example, if each additional year without smoking would put off the age at which diabetes was diagnosed by a year (i.e., constant increase in risk at all ages) a gradient of one would result. The statement that there is no temporal relationship between smoking and diabetes can be rephrased as the age distribution of diabetes onset would be unaffected

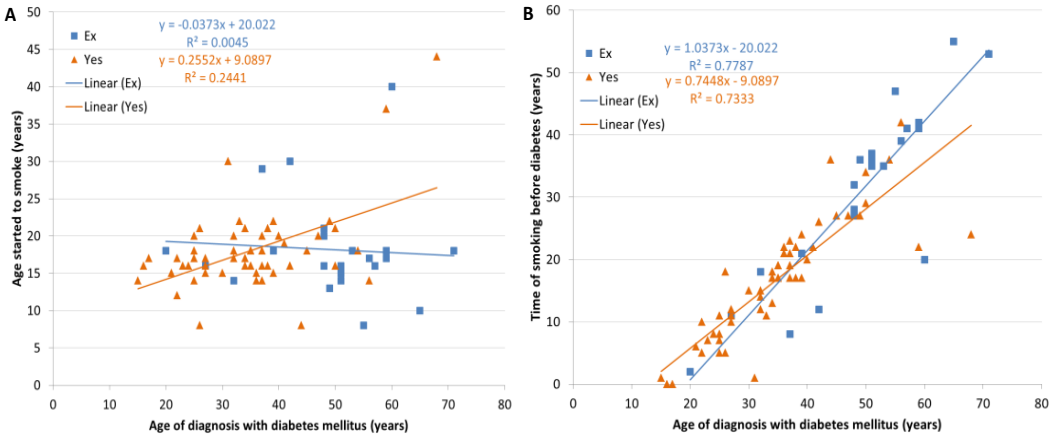
by the age distribution of smoking uptake. By definition, a plot of age at which smoking began against age of diabetes onset would be zero (a flat line).

As shown in Figure 19A, the time at which smoking begins makes little or no difference in the age of diabetes diagnosis. The gradient for smokers at the time of diagnosis with diabetes was just 0.255 and the R^2 value showed a weak correlation (0.244), for ex-smokers the gradient was even more clearly inconsistent with smoking increasing risk for all HP patients, in fact the gradient was weakly negative (-0.04) with no real evidence of association (R^2 <0.01).

An alternative way to look for a link between smoking and diabetes is to plot the time between starting smoking and being diagnosed with diabetes against the age of diabetes onset. For example, if every smoker develops diabetes exactly 10 years after starting to smoke, then an individual who develops diabetes at 40 years will have inevitably started smoking at 30, while an individual who develops diabetes at 50 will (equally inevitably) have started smoking at 40. For each age of diabetes onset, the time between starting smoking and diabetes onset will be 10 and therefore the gradient of the plot will be zero. In contrast if there is no association there should be a gradient of 1: as age of starting to smoke will be random for each age of diagnosis with diabetes. In Figure 19B a gradient close to 1 is seen for ex-smokers and 0.745 for smokers. In both cases the R^2 value suggests we can confidently assume age of diabetes diagnosis is not linked to duration of smoking exposure.

Smoking however, does not increase the risk of developing diabetes uniformly for all HP patients. The time at which smoking begins makes little or no difference in the age of diabetes diagnosis; there is no fixed period of smoking that causes diabetes (R_2 = 0.241), and age at diabetes onset is not related to age duration of smoking (R_1= 0.733).

No temporal relationship between smoking and diagnosis of diabetes



Gradient for ex smokers ≈ 0

Gradient for smokers < 0.3

Gradient for ex smokers ≈ 1

Gradient for smokers > 0.7

Figure 19. There is no temporal relationship between age of diagnosis of diabetes and age of smoking uptake.

A) Age of starting to smoke plotted against age of diabetes diagnosis. Gradient for Ex-smokers = 0, Gradient for smokers <0.3. B) Time from starting to smoke to age of diabetes diagnosis. Gradient for Ex-smokers = 1, Gradient for smokers >0.7.

6.4.3.7 Age of onset of pancreatitis and the diagnosis of diabetes

The relationship between age of onset of pancreatitis and age of diagnosis of diabetes is also weak (Figure 20 A and B) although the evidence for a temporal association (the gradient in Figure 21A is greater than in Figure 20A and the gradient in Figure 21B are smaller than Figure 20B).

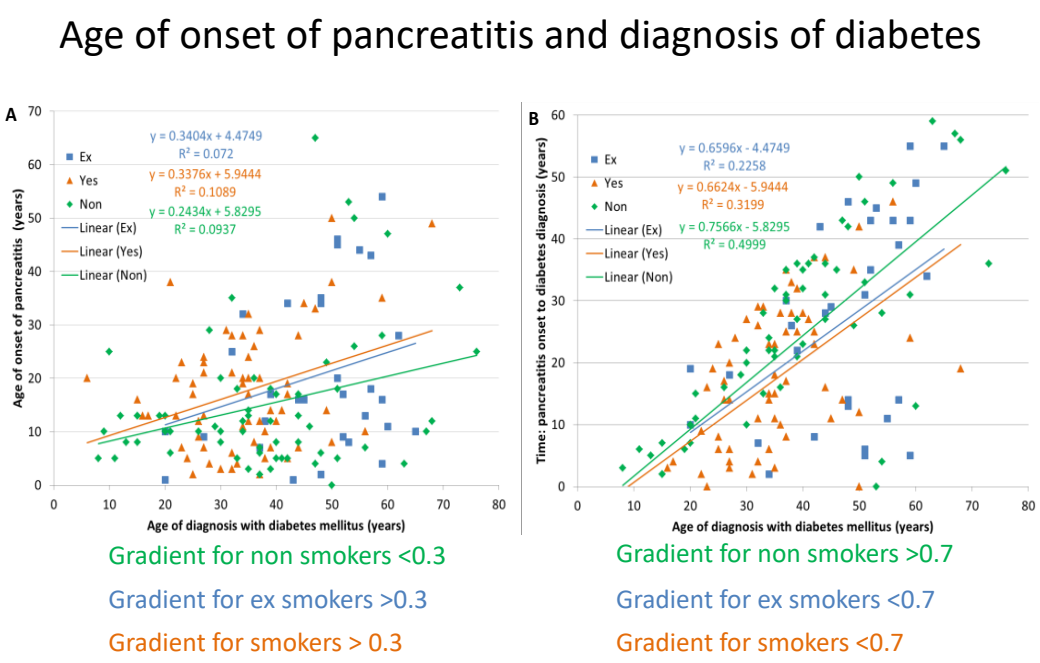


Figure 20. The relationship between age of onset of pancreatitis and diagnosis with diabetes.

A) Age of pancreatitis onset against age of diabetes diagnosis. B) Time from onset of pancreatitis to diabetes diagnosis

6.4.3.8 The temporal relationship between diagnosis of diabetes and PDAC

The temporal association between diabetes onset and pancreatic cancer seems stronger than the association between pancreatitis onset and diabetes (smokers R^2=0.69; non-smokers R^2= 0.588), as seen in Figure 21, with the exception being in ex-smokers where there does not seem to be a temporal relationship. However, these temporal associations (between diabetes and cancer) are difficult to prove given the small numbers involved.

There was no temporal relationship between the age at which smoking began and diagnosis of diabetes; there was no fixed period of smoking that caused diabetes (R^2 = 0.241) with duration of smoking giving a near linear relationship with age of diabetes diagnosis (R^2= 0.733, gradient ~1.0).

A weak relationship was seen between onset ages of pancreatitis and diabetes. A stronger relationship was demonstrated between diagnosis ages of diabetes and pancreatic cancer (smokers R^2=0.69; non-smokers R^2= 0.588).

Both diabetes [HR=2.34 (95% CI: 1.25, 5.98; P = 0.015)] and tobacco smoking [3.53 (1.42, 9.67); P = 0.0047] were independent risk factors for pancreatic cancer.

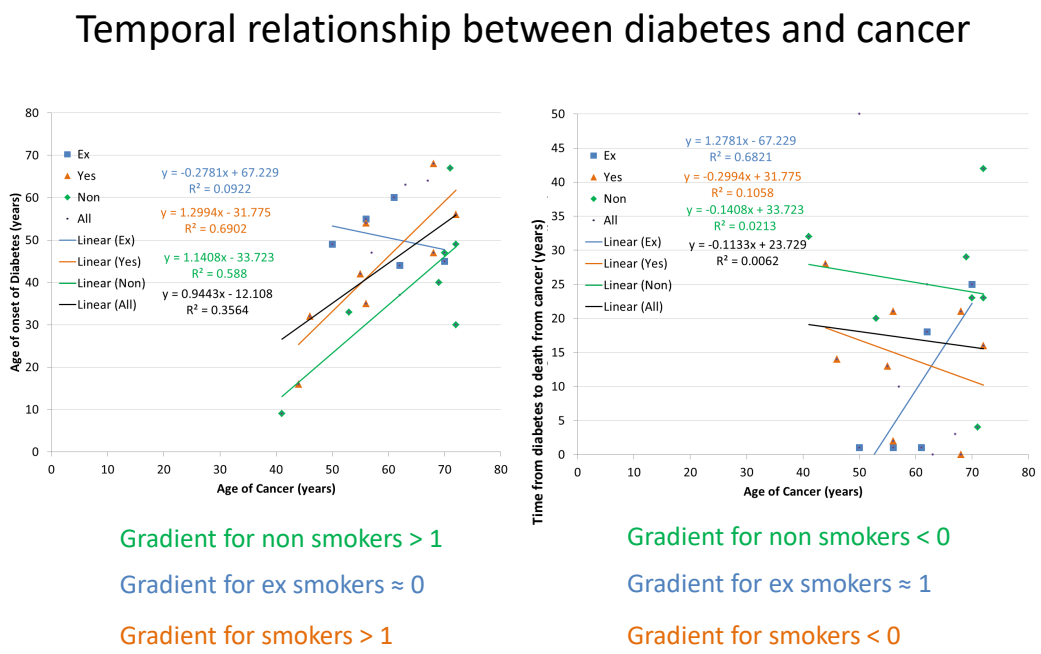


Figure 21. Evidence for temporal relationship between diabetes and cancer.

A) Age of diabetes diagnosis against age of death from cancer. B) Time from diabetes to diagnosis to death from cancer.

6.4.3.9 The Frailty model and sensitivity analysis

Using a gamma shared-frailty model to account for familial clustering both diabetes [hazard ratio 2.16 (95% CI: 1.46, 2.86; P = 0.015)] and tobacco smoking [4.20 (3.45, 4.96); P = 0.0001] were independent risk factors for

pancreatic cancer. Only smoking remained independently significant [3.96 (1.67, 11.52); P = 0.002] after when a sensitivity analysis taking diabetes diagnosis within one year of cancer as a symptom rather than a predisposing factor was applied.

Table 16. Extended Cox proportional hazard model including univariate and multivariate analyses.

Variable	Univariate analysis			Multivariate analysis		
	Coefficient (SE)	**Hazard ratio (95% CI)**	**p-value**	**Coefficient (SE)**	**Hazard ratio (95% CI)**	**p-value**
Smoking (vs No)						
Current	1.21 (0.418)	3.34 (1.47, 7.58)	0.0039	1.44 (0.386)	4.20 (3.45, 4.96)	0.0001
Ex	-0.08 (0.462)	0.92 (0.37, 2.28)	0.8600	-0.03 (0.446)	0.97 (0.10, 1.85)	0.5254
Diabetes onset	1.27 (0.337)	3.56 (1.84, 6.88)	0.0002	**0.77 (0.355)**	**2.16 (1.46, 2.86)**	**0.0150**
Duration of smoking	0.01 (0.010)	1.01 (0.99, 1.03)	0.2300	-	-	-
Gender (vs F)	0.05 (0.319)	1.05 (0.56, 1.97)	0.8800	-	-	-
Transmission (vs unknown)						
Maternal	0.01 (0.394)	1.01 (0.47, 2.19)	0.9800			
Paternal	0.00 (0.424)	1.01 (0.44, 2.31)	0.9900	-	-	-

Mutation (vs A16V)	0.28 (1.106)	1.32 (0.15, 11.53)	0.8000			
N29I	1.12 (1.078)	3.08 (0.37, 25.47)	0.3000			
NEG.ALL.HP	0.66 (1.054)	1.93 (0.24, 15.22)	0.5300	-	-	-
R122H						
Nationality (vs Other)	0.82 (0.824)	2.26 (0.45, 11.35)	0.3200			
Germanic	0.84 (0.810)	2.31 (0.47, 11.30)	0.3000			
Latin	0.44 (0.778)	1.56 (0.34, 7.18)	0.5700			
UK/Ireland						

Table 16 below shows the resulting hazard ratios (with corresponding confidence intervals) against thresholds (x) of 0–5 and 10 years between diabetes and diagnosis of cancer. The hazard ratios of the diabetes onset for follow-up less than 0, 1, 2, 3, 4, 5 and 10 years are 2.16 (from table above), 1.38, 1.19, 1.20, 1.04, 1.05 and 1.03 respectively. The most extreme estimate of 2.16 occurs when we assume that the onset of diabetes is not the first symptom of an undiagnosed pancreatic cancer, regardless of the length of time between these two events. However, as shown from following estimates, this estimate of 2.16 is sensitive to the above assumption with the hazard of pancreatic cancer at the occurrence of diabetes reducing to around 1 for all time periods between 1 and 10 years.

Table 17. Sensitivity analysis taking diabetes diagnosis within one year of cancer as a symptom rather than a predisposing factor.

x years	Variable	Multivariate analysis		
		Coefficient (SE)	Hazard ratio (95% CI)	p-value
1	Smoking (vs No)			
	Current	1.55 (0.464)	4.73 (3.82, 5.64)	0.0004
	Ex	0.06 (0.445)	1.06 (0.19, 1.94)	0.4438
	Diabetes onset	0.32 (0.333)	1.38 (0.72, 2.03)	0.1693
2	Smoking (vs No)			
	Current	1.57 (0.398)	4.82 (4.04, 5.60)	0.0000
	Ex	0.06 (0.424)	1.06 (0.23, 1.89)	0.4478
	Diabetes onset	0.18 (0.311)	1.19 (0.58, 1.80)	0.2865
3	Smoking (vs No)			
	Current	1.53 (0.406)	4.60 (3.81, 5.40)	0.0001
	Ex	0.02 (0.434)	1.02 (0.17, 1.87)	0.4787
	Diabetes onset	0.18 (0.313)	1.20 (0.59, 1.81)	0.2782
4	Smoking (vs No)			
	Current	1.55 (0.385)	4.73 (3.97, 5.48)	0.0000
	Ex	0.07 (0.429)	1.08 (0.25, 1.90)	0.4294
	Diabetes onset	0.04 (0.338)	1.04 (0.38, 1.71)	0.4482
5	Smoking (vs No)			
	Current	1.55 (0.398)	4.71 (3.93, 5.49)	0.0001
	Ex	0.07 (0.435)	1.07 (0.22, 1.92)	0.4400
	Diabetes onset	0.05 (0.332)	1.05 (0.40, 1.70)	0.4453
10	Smoking (vs No)			
	Current	1.58 (0.426)	4.83 (4.00, 5.67)	0.0001
	Ex	0.09 (0.440)	1.10 (0.23, 1.96)	0.4178
	Diabetes onset	0.03 (0.337)	1.03 (0.37, 1.69)	0.4623

6.5 Discussion

Hereditary pancreatitis is associated with a 40% lifetime risk of developing PDAC however, thankfully in real world terms this diagnosis effects very few individuals.

EUROPAC screens high risk patients including those with HP for PDAC on a research basis. The UK based EUROPAC screening of individuals during this study period yielded 5 CRLs (13%) at a rate of 1 CRL per 34 screening years follow-up. Two participants had positive PJMA and after referral to the pancreatic supra-regional MDT went on to have pancreatic resection. A main duct IPMN was identified in a further individual, however, between screening cycles this participant had developed metastatic breast cancer and sadly died prior to discussion at the MDT (screening continued whilst the aim of breast cancer treatment was curative as PDAC is associated with a significantly worse prognosis).

Unfortunately, we observed a high level of screening protocol deviation across the UK centres which resulted in 2 cases of PDAC in the cohort being missed. These were the only PDAC cases to be diagnosed within this study period. It is also important to remember that screening is not without risk. False negative results can trigger a slippery slope of increasingly invasive investigation, and as demonstrated in this study, if protocols are not strictly followed then screening can do more harm than good.

Initial EUROPAC screening study results by Howes et al (21, 243) estimated the PDAC rate in HP as one per 703 person years follow-up. Moving forward, despite the elevated PDAC risk with HP, EUROPAC was only aware of around 1 new PDAC diagnosis every 2 years within our large cohort of registrants. This raises the question as to why when dealing with such a high-risk population are we seeing so few cases of PDAC developing? Why are more cases not being identified through screening? These questions lead us to ask are we actually screening the right high-risk individuals and what can we do to improve screening outcomes?

6.5.1 Improving screening outcomes

6.5.1.1 Prevention of protocol deviations

The first step is to mitigate any further protocol deviations in the ongoing screening programme. If screening protocols are not strictly adhered to then potentially, they can cause more harm than good and the benefits to participants are negated.

UK based screening centres were contacted and or visited by the EUROPAC team and given a refresher on the approved screening protocol was provided. Screening centres were requested to forward on all results of screening investigations for review

by the EUROPAC screening committee even if they were deemed ‘normal’ by the local clinical team.

The paramount importance of continued patient education and maintenance of channels of communication with participants was recognised. Many participants enrol after a family member has died of PDAC and anxiety around their own personal risk is heightened. Over time this may wain or become less of a priority and so engagement and compliance with the screening protocol maybe affected.

Participants should also have their clinical eligibility for continued screening regularly reviewed. The clinical situation may change, the participant may receive other diagnoses that they do not feel are relevant to the EUROPAC screening study but may affect their appropriateness for treatment if PDAC was to be diagnosed. For example, they may no longer be a candidate for pancreatic resection or even suitable for life-prolonging palliative chemotherapy (if unfit for surgery). These decisions are very challenging and should be reviewed on a case-by-case basis.

6.5.1.2 Refining high risk individuals – risk stratification

Despite all the above, the yield of PDAC pick up in the screening cohort is low. Therefore, we must question whether we are targeting screening at ‘the right’ high risk individuals. There is a need to further stratify risk within high-risk groups like HP. Both smoking and diabetes have been identified as key features in further risk stratification. Lowenfals et al (189) demonstrated that smoking is related to PDAC risk and our data confirms this. We have also demonstrated that the development of DM is a risk factor for PDAC. The challenge faced here with further risk stratification is that smoking, and diabetes are competing risk factors for PDAC. In the EUROPAC HP cohort, those who were smokers developed DM more frequently than those who did not smoke. An interesting statistical anomaly was demonstrated in these data where ex-smokers developed diabetes later than are current smokers. There is a good reason for this; to have developed DM after starting and stopping smoking you must have reached a certain age, and that accounts for the observed lag. Once this lag has been accounted for the accumulation observed in ex-smokers was roughly as rapid as it is for the current smokers. So unlike with cancer risk, giving up smoking does not prevent you from developing DM. One could hypothesise that smoking tobacco causes a form of irreversible damage or change within the pancreas that leads to that person being

more likely to develop diabetes much later. This presents challenges for the development of statistical models.

Univariate analysis identified current smoking as a risk factor for developing PDAC in individuals with HP. Diabetes mellitus was also identified as a risk factor PDAC development. On multivariate analysis current smoking is still highly significant. Diabetes remains significant however, the level of significance is reduced. Diabetes is a complex disease in terms of pancreatic cancer. Individuals can develop diabetes as a manifestation of PDAC, and diabetes is also a risk factor for the development of PDAC. Within the sensitivity analysis, when those patients who have been diagnosed with DM within a year of PDAC were eliminated or censored, the statistical significance within this multivariate analysis was lost. Therefore, we must accept that currently we do not have any effective models for risk stratification in term of diabetes and smoking yet but the EUROPAC group continue to work on the further development of such models.

6.5.1.3 Modifications to the HP screening protocol

These data presented here led to improvements in the EUROPAC recruitment processes and modifications to the EUROPAC screening protocols. EUROPAC recruitment data collection now enriches for epidemiological and demographic data we have already shown to be important in the future analysis of PDAC risk and stratification of risk.

EUROPAC secondary screening protocol for pancreatic cancer have now been modified to include two potential approaches to screening: relaxed vs intense screening. Following recruitment and consenting to the secondary screening study, an individual's smoking status and diabetes status is considered and used to stratify their personal PDAC risk. The frequency of screening cycles is influenced by individuals smoking and DM status in addition to other factors which may make them more high risk. The modified EUROPAC screening protocol in summarised in Figure 22.

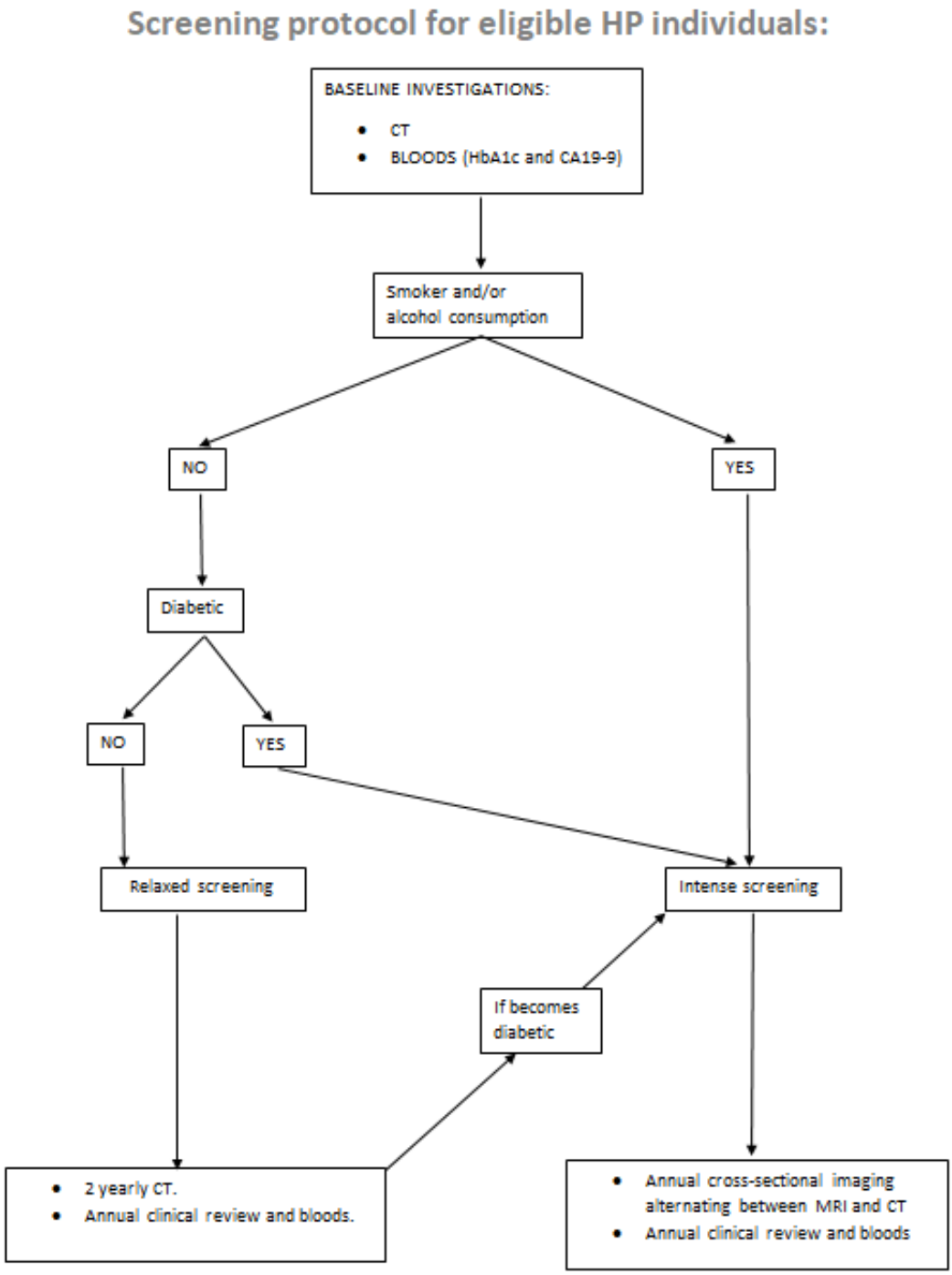


Figure 22. The modified EUROPAC screening protocol for HP as per 2021.

6.6 Conclusion

We believe the EUROPAC protocol works, however it must be strictly adhered to. If there is any doubt that the protocol could not or would not be followed properly then we would advise not initiating screening because there is the potential to cause more harm than good, as demonstrated with the 2 missed PDAC cases here. Participants clinical eligibility should also be regularly reviewed.

We need more adjuncts to help improve screening and stratification of risk. Within the EUROPAC HP population tobacco smoking and diabetes mellitus are competing risk factors for the development of PDAC which has influenced the outcome of the sensitivity analysis. This supports the hypothesis that people with HP could be sub-classified into those individuals at high risk of developing diabetes mellitus, and those who are at lower risk. Smoking is a modifiable factor that causes low risk individuals to become high risk for developing diabetes and increases risk of pancreatic cancer.

Therefore, there is a need for the further development of effective risk stratification models which successfully factor in these competing risk factors.

In the meantime, clinicians should educate HP patients at the earliest opportunity to never smoke.

Chapter 7: Identification of cystic lesions by secondary screening of familial pancreatic cancer kindreds is not associated with the stratified risk of PDAC

7.1 Introduction

There has been a global shift towards early detection of PDAC to improve outcomes supported by many international organisations. The best survival can be achieved when precursor lesions are identified and resected before they develop into invasive malignancy, or in small and early malignancies.

Even in the event of a locally advanced, borderline resectable, or even unresectable tumour at diagnosis, lower tumour burden (smaller tumours) has less chemoresistance making chemoradiotherapy more effective. However, if screening for pancreatic cancer is to be feasible, then screening should take place within a specially selected, highly enriched population of individuals who have a high prevalence of pancreatic cancer diagnosis. This increases the rate of detection and reduces false-positive results. Currently screening for PDAC is undertaken on a research only basis in populations with autosomal dominant predisposition for pancreatic cancer (221).

Screening for PDAC in other high-risk groups such as hereditary pancreatitis has already been covered. Here we will focus on families with inherited risk and cystic lesions of the pancreas.

7.1.1 Pancreatic cystic lesions

Pancreatic cystic lesions are common, with a prevalence of around 8% in those aged over 70 years (244). Increasingly pancreatic cystic lesions are identified incidentally on imaging performed for other indications with estimations suggesting 2.6 cysts identified per 100 patients imaged per year (95, 245). The most common pancreatic cyst is a pseudocyst (a fluid collection surrounded by inflammatory / fibrous tissue with no epithelial lining) (246). Pseudocysts are a common sequalae of acute and chronic

pancreatitis and often seen as a radiological outcome measure of inflammatory activity in CP. Pseudocysts have no malignant potential and have no association with the development of PDAC.

The remaining pancreatic cysts generally can be subclassified into serous cystic lesions and mucinous cystic lesions. Additional rare exceptions include cystic adenocarcinomas and cystic neuroendocrine tumours. Serous cystadenomas are common cystic lesions which have no malignant potential and have a strong association with mutations in VHL. Mucinous cystic lesions secrete fluid rich in mucin and are associated with an increased risk of malignant transformation. They include Intraductal Papillary Mucinous Neoplasms (IPMNs) and mucinous cystic neoplasms (MCNs).

The types of pancreatic cysts along with their malignant potential are described below.

Table 18. Types of pancreatic cysts and their malignant potential

Pancreatic cyst	Malignant potential
Pseudocyst	None
True cysts	
Mucinous	
Intraductal papillary mucinous neoplasm (IPMN)	Low to high
Mucinous cystic neoplasm (MCN)	Moderate to high
Nonmucinous	
Serous cystic neoplasms (SCN)	None
Solid pseudopapillary neoplasms (SPN)	Moderate
Cystic pancreatic endocrine neoplasms (CPEN)	Moderate

7.1.2 Pre-cursor lesions

Pancreatic cystic lesions with the highest malignant potential are the mucinous cystic lesions IPMNs and MCNs. These make up two of the three precursor lesions for PDAC, with the third being pancreatic intraepithelial neoplasia (PanIN).

The Baltimore consensus meeting in 2014 revised the recommendations and assessments of pancreatic cancer precursor lesions including pancreatic intraepithelial neoplasia (PanIN), intraductal papillary mucinous neoplasm (IPMN),

mucinous cystic neoplasm. Classification of precursor lesions were modified to allow improved concordance and alignment of outcomes. A two-tiered system for all precursor lesions was proposed which differentiate purely between low vs high grade dysplasia (247). The term high grade dysplasia was reserved purely for carcinoma in situ situations.

7.1.2.1 Pancreatic intraepithelial neoplasia

Pancreatic intraepithelial neoplasia (PanIN) describes proliferative lesions in the pancreatic ducts that represent precursors to invasive malignancy. PanIN has been found in up to 30% of autopsy specimens, with an increased incidence in patients with PDAC (245-247). PDAC most frequently arises from pancreatic intraepithelial neoplasia (PanIN) in the stepwise progression from intraepithelial to invasive pancreatic neoplasia. PanIN display several morphological changes as summarised in Fig 23. Initially, columnar, mucinous epithelium appears, followed by increasing architectural disorganisation and nuclear atypia as the stage increases. Finally, high-grade PanIN transforms into PDAC with evidence of areas of invasion beyond the basement membrane. These changes are seen in parallel with an accumulation of genetic changes supported by molecular profiling studies, which are thought to be initiated by the activation of KRAS (K-RAS2 oncogene mutations). A greater number of genetic alterations are seen in higher grade PanIN, with CDKN2A, p53 and SMAD4 mutations becoming more prevalent as dysplasia progresses from low grade to high grade until it becomes invasive carcinoma (248). Interestingly, the genetic mutation sequences observed vary in different PDAC precursor lesions. K-RAS mutations are common in IPMN (40-65%), along with activating GNAS mutations and inactivation of RNF43 (249). These differing genetic events may partly explain the minor differing prognoses seen in tumours arising from different precursors.

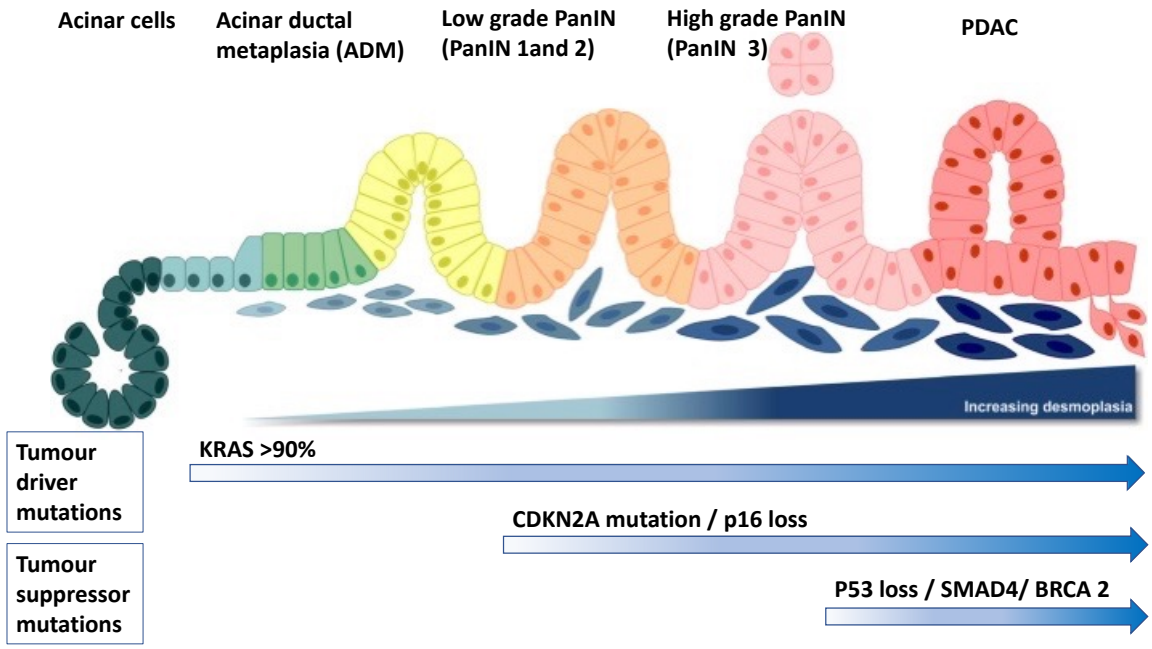


Figure 23 The development of PDAC from PanIN showing the morphological changes from low-grade, high-grade dysplasia, then invasive malignancy, and the associated accumulation of genetic mutations.

PanIN's are classified according to the 2014 Baltimore consensus meeting as low grade (historically PanIN 1a, PanIN 1b and PanIN 2) and high grade PanIN 3. Only carcinoma type lesions / high grade dysplasia type lesions are considered PanIN 3 (247).

Table 19. Summary of the PanIN classifications adapted from Basturk et al (247)

Pancreatic intraepithelial neoplasia classification		
Oldest terminology	**WHO 2010 classification**	**Baltimore consensus meeting 2014**
Mucinous (goblet cell) metaplasia, flat duct lesion without atypia, mucinous ductal hyperplasia, simple hyperplasia, mucinous cell hyperplasia, flat duct hyperplasia, nonpapillary epithelial hypertrophy	PanIN 1A	Low grade PanIN
Papillary hyperplasia, papillary duct lesion without atypia, ductal hyperplasia, adenomatoid hyperplasia	PanIN 1B	

Atypical hyperplasia, papillary duct lesion with atypia, low grade dysplasia, moderate dysplasia	PanIN 2	
Carcinoma in situ, intraductal carcinoma, high grade dysplasia, severe dysplasia	PanIN 3	High grade PanIN

Early detection of PanINs presents an opportunity to prevent patients developing PDAC however, PanINs are currently only detectable on histopathology obtained from surgical resection. No current cross sectional imaging modality or EUS technique can identify them. Molecular analysis of pancreatic juice may help to identify individuals at higher risk of developing PDAC, however, there is emerging evidence against the traditional progression model. A catastrophic process of cancer development associated with Acinar Ductal Metaplasia and lobular atrophy, independent of PanIN (250, 251) fits with observations in familial pancreatic cancer.(252) Atypical Flat Lesions may be positive screening results on this basis (253), but unfortunately these are only likely to be identified after the pancreas has been resected.

7.1.2.2 Mucinous cystic neoplasms

MCNs classically are found most in females in their 5th decade of life. Most (95%) occur in the pancreatic body or tail and the majority (65%) are symptomatic, presenting with abdominal pain, back pain, fullness, or acute pancreatitis for example. The remainder are diagnosed incidentally on cross sectional imaging for other indications. In 2000 the WHO described MCNs as an epithelia neoplasm which lacks communication with the pancreatic duct and contains ovarian stroma. The most recent WHO update subclassifies MCNs as: MCN with MCN with low- or intermediate-grade dysplasia (previously called mucinous cystadenoma), MCN with high grade of dysplasia (previously called mucinous cystadenocarcinoma) non-invasive, and MCN with an associated invasive carcinoma—if component of invasive carcinoma present (254).

Reported incidence of malignancy in MCNs varies greatly across the literature. Based on the most contemporary studies, the incidence of malignancy is between 10-39% (255-259). Like IPMNs, MCNs as mucinous pancreatic lesions have a high CEA and mucinous cytology in the cyst fluid (260). Markers of potential malignant transformation

has been investigated. Raised serum carcinoembryonic antigen (CEA) and carbohydrate antigen 19-9 (CA 19-9) levels have a high predictive value for malignancy (70-100% PPV) (256), Cyst fluid CEA level > 192ng/ml, Cyst size, and presence of mural nodules/ solid components/ duct dilatation are all considered concerning features for potential malignant transformation. A multicentre Asian study confirmed no malignancy in MCNs 3cm or smaller, with most malignant lesions being found in MCNs greater than 5cm (258). Mural nodules are most concerning if > 1cm in size (258). European and Japanese consensus guidelines both recommend that MCN's should be resected if the patient is fit enough for surgery ((218, 261). Surgery would be in the form on an oncological resection i.e distal pancreatectomy with splenectomy of MCNs of the body/tail and a PPPD for a head/neck MCN. Minimally invasive techniques and spleen and parenchymal sparing surgery is controversial but can be considered on an individual basis.

7.1.2.3 Intraductal Papillary Mucinous Neoplasms

Intraductal Papillary Mucinous Neoplasms (IPMNs) are an intraductal proliferation of mucin-producing cells arranged in papillary formations which can occur in the main pancreatic duct or branch ducts (262). IPMNs are the most encountered precursor lesions and account for a quarter of all cystic lesions of the pancreas. IPMNs are more common with increasing age (263-265), associated with the presence of diabetes mellitus (266), and CP (266, 267). Around 8% of all PDACs arise from IPMNs (268).

Most IPMNs and pancreatic cystic neoplasms are found incidentally during routine cross-sectional imaging for other indications and are thus asymptomatic. They can cause abdominal/back pain, vomiting, and weight loss. Around one fifth of IPMNs present with an attack of acute pancreatitis caused by thick mucin obstructing the pancreatic duct (269-272). Presentation with jaundice is a sinister feature and is often associated with invasive malignancy.

IPMNs are classified anatomically, according to the grade of dysplasia (WHO), and according to their cellular morphology. The original IAP 2006 'Sendai" consensus guideline classified IPMNs based on their site of origin. A main duct (MD-IPMN) had segmental or diffuse dilatation of the main pancreatic duct (MPD) in the absence of distal obstruction. Branch duct (BD-IPMN) has mucinous pancreatic cyst

communicating with the pancreatic ductal system in the absence of MPD dilatation (273). The 2012 Fukuoka guidelines revised the 2006 IAP Sendai document and made some important additions and refinements. Mixed-type IPMNs, defined as lesions with features of both MD and BD IPMNs were classified together with MD-IPMNs due to their similar malignancy risk (around 45%) (274). The upper limit of a normal MPD was also defined as 5mm (in the absence of obstruction), and all cysts of >5mm diameter in communication with the MPD were classified as BD-IPMNs.

All IPMNs are dysplastic, with the epithelial lining exhibiting varying degrees of dysplasia, before progressing to invasive carcinoma. The WHO 2010 classification of IPMN (adopted in the Fukuoka 2012 guidelines) categorises IPMNs into; low, intermediate, and high-grade dysplasia, followed by invasive cancer.

IPMNs can be further classified by histological type including gastric, intestinal, pancreaticobiliary or oncocytic based on cellular morphology and mucin (MUC) gene expression and tissue architecture. These subtypes may impact on prognosis as summarised in table 20.

Table 20 Histological classification of IPMNs based on cellular morphology.

IPMN subtype	**Features**	**% of IPMN**	**% invasive progression**	**5-year survival**
Gastric	Associated with BD-IPMN Non-invasive low-/moderate-grade neoplasms No invasion	43-63	10	>90%
Intestinal	Associated with MD-IPMN Invasive colloid carcinomas	18-36	40	>80%
Oncocytic	Affected significantly younger patients Invasive oncocytic carcinomas	7-18	50	>80%

Pancreatobiliary	More common in women, older age group Advanced disease (stage IIb)	1-8	68	>50%

MD-IPMNs have the greatest malignant potential with reports of malignancy in 23-57% whilst BD-IPMNs have a lower risk reported in the region of 0-31% (273).

There is some controversy over whether IPMNs develop into PDAC, invasive IPMN being a separate form of malignancy, with a better prognosis (275). PDAC do develop in patients with IPMN, sometimes after the IPMN have been successfully resected (276). Suggesting association beyond the risk of IPMN and cancer that goes beyond simple progression.

Currently there are no standalone serum or cytological tests that can distinguish between IPMNS of low or high risk of malignant transformation. CA19-9 is the only validated serum biomarker in PDAC. Its role is currently limited to clinical follow-up post pancreatic resection. The role of CA 19-9 in predicting malignant transformation in IPMN was evaluated in a metanalysis. It was found to be very specific, but lacked the required specificity to be utilised as a stand-alone diagnostic test (277). CEA is often raised in patients with gastrointestinal cancers and a study from Heidleberg showed that CA 19-9 in conjunction with CEA were raised in 80% of patients with invasive malignancy compared to 18% with dysplasia (278). The presence of KRAS mutations are useful to differentiate between IPMN and MCN in cases of diagnostic uncertainty, but have not been shown to distinguish between dysplasia and invasive malignancy in IPMN (279).

Radiological and clinical features in addition to mutational profiles from cystic fluid can help differentiate between IPMNs with a low or high malignant potential. Full radiological imaging of IPMNs typically consists of a combination of pancreas protocol CT and MRCP. EUS and PETCT are also useful adjuncts. Pancreas CT provides information on relationship/involvement of other structures, on calcification, and accurately identifies worrisome features. MRCP is an excellent imaging modality for evaluating cystic lesions. It provides the most detailed information on the pancreatic ductal system and is often able to distinguish between BD-IPMN and MCN. MRCP is the investigation of choice for the surveillance of IPMNs and other cystic lesions due to the low ionising radiation level. EUS is very good for assessment of worrisome

features and has the added advantage of facilitating histological/cytological sampling of the lesion during the same sitting. The role of PETCT in the assessment of IPMNs remains unclear. Some studies have reported accurate differentiation between benign IPMNs and those harbouring malignant transformation (non-invasive and invasive) (280), however the rate of false positives was high.

7.1.3 Management of IPMNs

Ease of access to routine cross-sectional imaging has led to increased prevalence of pancreatic cystic lesions, particularly IPMNs being identified incidentally. This poses new challenges in balancing the need for earlier identification of precursor lesions and cancer prevention with the risks associated with over investigation and surgical over treatment.

Recommendations on the management, surveillance, and surgical resection of IPMNs and other PCNs are provided by 3 main international guidelines. The 2015 American Gastroenterological Association (AGA) (281); the 2017 revision of the International Association of Pancreatology (IAP) (282); and the 2018 revision of the European Study Group on Cystic Tumours of the Pancreas (European) (283). There are controversies between the three documents. They differ in their recommendations for surgical resection, management of BD-IPMN, surveillance protocols and criteria for ceasing surveillance. The quality of the evidence used in the guideline development process is low and so many of the recommendations are based on expert and consensus opinion.

Absolute indications shared across guidelines included MD-IPMN, with concerning features such as obstructive jaundice, enhancing mural nodules, and positive cytology (281, 282, 284, 285). The management of BD-IPMNs and the role of cyst surveillance were less clear.

The 2006 IAP Sendai guidelines established the presence of a MD-IPMN as an absolute indication for surgery due to the high risk of invasive malignancy. For BD-IPMN where the malignancy risk is lower resection was recommended for lesions >3cm, or in the presence of mural nodules, MPD dilatation without obstruction, or abnormal cytology on sampling.

The 2012 Fukuoka revisions to the Sendai guidelines introduced the concept of 'high risk stigmata' (table 21) where the recommendation was for resection in appropriately fit patients, and worrisome features whose presence should prompt further evaluation with EUS to identify mural nodules, define cyst relation to main pancreatic duct and obtain tissue for cytological analysis. Following more detailed evaluation, patients with confirmed absence of high-risk stigmata would be offered further surveillance.

Table 21 2012 Fukuoka classification of worrisome features and high-risk stigmata in IPMNs.

Worrisome features	**High risk stigmata**
MPD 5-9mm	MPD ≥ 10mm
Non-enhancing mural nodules	Enhancing solid component in cyst
Abrupt change in calibre of main duct with atrophy of the distal pancreas	Jaundice in patient with cystic lesion in the head of the pancreas
Cyst ≥ 3cm	
Cyst wall thickening and enhancement	
Recommendation: Further evaluation with EUS	Recommendation: Resection

The IAP Fukuoka and the European guideline have concordance on many points. The European guideline document also supports a conservative approach to managing BD-IPMN, but of not stratifying the risk of progression according to (amongst other factors) familial history of pancreatic cancer (284). The American Gastroenterological Association guidelines remain controversial due to the suggested limit to the length of surveillance of BD-IPMN. They essentially enforce the idea of BD-IPMN as lesions that can, in most cases, be safely left in situ. Concern has been raised over the potential of missing malignancy if the guidelines were followed and cysts which have been stable for 5 years are discharged from follow up and surveillance.

The Fukuoka guidelines are regarded by many as having gone through the most rigorous guideline development process, on an international platform. Many centres base their surveillance protocols on these guidelines that's to their clear, logical, and

clinically relevant presentation which in essence suggested surveillance of BD-IPMN and resection of MD-IPMN.

The absolute and relative indications for surgery according to each guideline are summarised below.

Table 22 A summary of the recommendations for the absolute and relative indications for surgery for pancreatic cystic lesions from the 3 main international guidelines adapted from van Huijgevoort et al (278).

Guideline	Cyst	Absolute indication for resection	Relative indications for resection
2015 AGA (281)	MCN	Any MCN	-
	IPMN	PD ≥5mm* AND solid component to cyst OR positive cytology	-
2017 IAP (282)	MCN	Any MCN	
	IPMN	• PD dilatation ≥10mm • Jaundice • Enhancing mural nodule ≥5mm • Positive or suspicious cytology	• Growth rate ≥5 mm over 2 years • Increased levels of serum CA19-9 • PD dilatation between 5 and 9 mm • Cyst diameter ≥30 mm • Acute pancreatitis (caused by IPMN) • Enhancing mural nodule (<5 mm) • Abrupt change in diameter of PD with distal pancreatic atrophy • Lymphadenopathy • Thickened or enhancing cyst walls
2018 European (284)	MCN	• Cyst diameter ≥40 mm • Enhancing mural nodule • Symptoms (that is jaundice (tumour-related), • acute pancreatitis (caused by MCN), new-onset diabetes mellitus)	-

	IPMN	•Positive cytology for malignancy or high-grade dysplasia • Solid mass •Jaundice (tumour-related) •Enhancing mural nodule (≥5 mm) •PD dilatation ≥10 mm	• Growth rate ≥5 mm per year • Increased levels of serum CA19-9 (>37 U/mL)c • PD dilatation between 5 and 9.9 mm • Cyst diameter ≥40 mm • New-onset diabetes mellitus • Acute pancreatitis (caused by IPMN) • Enhancing mural nodule (<5 mm)

* On MRI or EUS

7.1.3.1 IPMNs in high-risk individuals

Identifying a BD-IPMN during screening raises the question of whether the lesion should be managed according to standard guidelines, or whether this represents a consequence of the genetic predisposition for cancer and so could merit immediate resection. This will depend on the nature of the genetic predispostion, syndromes such as Familial Adenomatous Polyposis (FAP) predispose to cancer because they predispose to precursor lesions. These precursor lesions, albeit more commonly found, are not greatly more prone to progression than similar lesions found in individuals without genetic predisposition for cancer (286). In contrast, Lynch Syndrome (or Hereditary Non-Polyposis Colorectal Cancer, HNPCC) increases the risk of pre-cursor lesions progressing (287), so lesions are less likely to be found but are much more worrisome if identified. Naturally, if lesions are not related to the genetic predisposition for cancer then they will neither be more frequent nor more aggressive.

Multigenic cancer predisposition will give heterogeneous risk with only particular combinations of alleles passing a threshold that would allow predictable development of malignancy, even if all family members have some small elevated risk (288). The combination of alleles responsible for specific cancer cases will be unlikely to be seen again in the same family, so prospective risk would be too low to justify cancer screening. Effective screening requires a single mutant gene that confers the bulk of risk, although this may well be context specific (some genetic backgrounds giving high penetrance and some low penetrance) in such a situation, family members who are non-carriers must be assumed not to be at any elevated risk. This form of inheritance

is rare but can be seen in various syndromes including Breast-Ovarian Cancer, where *BRCA1* or *BRCA2* mutations are causative; Peutz-Jegher's (PJS), where *STK11* mutations are causative; Lynch Syndrome, where mismatch repair genes confer risk; and Familial Atypical Multiple Mole Melanoma (FAMMM), which can be due to *CDKN2a* mutations. Binary risk, based on autosomal dominant predisposition will be assumed in this work.

PJS is associated with IPMN and mutations causing inactivation of the STK11 gene are thought to predispose to malignant progression in IPMN (289, 290). Individuals from FPC kindreds have been shown to develop BD-IPMNs during screening and that this could be concerning for the presence of high-grade dysplasia or invasive malignancy. Therefore it may be appropriate to make an argument to offer these 'individuals at risk' risk reducing surgery in the form of total pancreatectomy (291)

7.1.4 Genetic predisposition to PDAC

Those with a family history of PDAC and those with germline mutations in specific genes are associated with a greater risk of developing the condition (292-294). Genetic risk in PDAC however, is multifactorial. Many genetic mutations have been shown to be associated with PDAC however these mutations are found as frequently in sporadic PDAC as they are in those with a strong family history (295) and most families with a strong history of PDAC do not have the mutations known to be associated with pancreatic cancer (292, 294-297).

Some hereditary conditions cause an increased risk of PDAC and mutations in certain genes (BRCA2, CDKN2A, STK11, PRSS1, PALB2) are also associated with the disease at variable penetrance.

Hereditary pancreatitis caused by a mutation in the PRSS1 gene is known to increase lifetime risk of PDAC 40-fold. HP has been discussed above.

7.1.4.1 BRCA2 and Hereditary breast and ovarian cancer syndrome

BRCA2 mutation carriers constitute the largest group of mutation carriers at risk for PDAC (292). The PDAC risk in this group is between 3.5-10%, depending on number of affected family members (298). The PDAC risk would appear to be context specific as not all carriers in a BRCA2 family will develop PDAC. Pandharipande et al used

MRI based simulation screening model for PDAC in BRCA2 families and demonstrated a small life expectancy gain with screening, which was eliminated with a slight increase in surgical mortality rate (>2.3%) (299, 300). The author's recommendation was to restrict screening to BRCA2 mutation carriers with at least 2 FDRs with PDAC, whereas EUROPAC offers screening to BRCA2 mutation carries with on case of PDAC on kindred.

Hereditary breast and ovarian cancer syndrome is characterised by an autosomal dominant predisposition for breast or ovarian cancer. Many families with HBOC also have mutations in BRCA2. Families with BRCA2 mutations have an increased incidence of pancreatic ductal adenocarcinoma and breast or ovarian cancer and are thus classified as having both HBOC and FPC (301).

7.1.4.2 Familial atypical multiple mole and melanoma (CDKN2A)

Familial atypical multiple mole and melanoma (FAMMM) is an autosomal dominant syndrome with a subset of patients with this syndrome harbouring mutations in *CDKN2A* (the gene encoding p16 tumour suppressor protein), which are frequently found in sporadic pancreatic cancer (302). Some FAMMM families also have cases of PDAC and create a subgroup called FAMMM pancreatic cancer. The estimated cumulative risk of developing PDAC in CDKN2A is 17% (303).

7.1.4.3 Hereditary non-polyposis colorectal cancer (HNPCC)

Hereditary non-polyposis colorectal cancer (HNPCC) or Lynch syndrome is associated with mismatch repair mutations that have been linked to pancreatic ductal adenocarcinoma. Not all HNPCC families are at increased risk of PDAC. Those families with HNPCC1 have an increased risk of colorectal cancer, whist HNPCC2 families are associated with the development of other cancer types including PDAC. Both subtypes of HNPCC include families with no identified causative mutation.

7.1.4.4 Peutz-Jeghers' syndrome (STK11)

Peutz-Jeghers' syndrome (PJS) is an autosomal dominant disorder with increased risk of multiple cancers. The phenotype comprises hamartomatous gastrointestinal polyps, and oro-buccal mucosal pigmented macules most of whom have an STK11 gene mutation with a very high pancreatic with cancer risk (304). A meta-analysis of 210 patients found the relative risk (95% confidence interval, CI) for all cancers was 15.2

(2, 19) % with an average age of onset of malignancy of 41 years, compared with over 60 years for the general population (305). The cumulative risk for all cancer was 93% from age 15 to 64 years old. A statistically significant increased relative risk (95% CI) was found for oesophagus (57; 2.5, 557%), stomach (213; 96, 368 %), small intestine (520; 220, 1306%), colon (84; 47, 137%), pancreas (132; 44, 261%), lung (17.0;5.4, 39%), breast (15.2;7.6, 27%), uterus (16.0; 1.9, 56%), ovary (27; 7.3, 68%), but not testicular or cervical malignancies. EUROPAC offers screening to individuals with PJS from the age of 20 years (304).

Whilst HBOC, HNPCC, FAMMM and PJS are all associated with PDAC, none of these syndromes are characterised by multiple occurrences of pancreatic ductal adenocarcinoma. In contrast, familial pancreatic cancer is a syndrome that is defined as having multiple first-degree relatives with pancreatic ductal adenocarcinoma, exclusive of other cancer syndromes (306). Familial pancreatic cancer can be associated with mutations in BRCA2 and possibly CDKN2A or even PALB2 (a breast cancer susceptibility gene with a protein product that enables BRCA2 anchorage to nuclear structures), but in most cases the causative mutation is unknown.

7.1.5 Familial pancreatic cancer (FPC)

A Familial Pancreatic Cancer (FPC) family is defined as having at least 2 first degree relatives (FDRs), or 3 or more second degree relatives, with pancreatic cancer. Despite pancreatic cancer being relatively uncommon, clusters of cases will occur by chance. If the cluster is random then prospective incidence of pancreatic cancer would be equivalent to the general population. A family history will make it more likely that an individual is carrying some high-risk allele of a gene, but most people with a family history will not have any genetic predisposition and instead family members may share some environmental risk factor. Stratification of risk is based on a family history, the greater the chance that a family has autosomal dominant genetic predisposition, the greater the risk estimate. Klein et al have shown that the risk of PDAC in an individual with 2 FDRS affected is 6.4 fold greater than someone with no affected FDRs (8-12% lifetime risk), whereas an individual with 3 FDRs can be estimated to have a 32-fold increased PDAC risk (40% lifetime risk) (307).

Risk can be further refined if an individual has undergone specific genetic testing to identify mutations in known pancreatic cancer susceptibility genes. Several germline

gene mutations have been identified as increasing PDAC risk (222). The relative risk of PDAC associated with each gene mutation is summarised in table 23.

Situations where a single mutant gene that confers the bulk of the cancer risk and those family members who are non-carriers should be assumed to be at no greater risk than the general population are rare but can be seen in hereditary Breast-Ovarian Cancer syndrome, where *BRCA1* or *BRCA2* mutations are causative; Peutz-Jegher's (PJS), where *STK11* mutations are causative; Lynch Syndrome, where mismatch repair genes confer risk; and Familial Atypical Multiple Mole Melanoma (FAMMM), which can be due to *CDKN2a* mutations.

Table 23. Inherited gene mutations associated with PDAC.

Hereditary tumour predisposition syndromes	Gene mutations	% of Presumed FPC Families	Relative Risk	Risk at 70 years of age (%)	Screening offered on research basis
None	None	-	1	0.5	No
Peutz–Jeghers syndrome	*STK11/LKB1*	<1	132	30-60	Yes
Hereditary pancreatitis	*PRSS1*	0	50-80	25-40	Yes
FAMMM	*p16/CDKN2A*	1	20-34	10-17	Yes
Hereditary nonpolyposis colon cancer (HNPCC)	MSH2, MLH1, MSH6, etc.	<1	8	3.7	Yes
Hereditary breast ovarian cancer	*BRCA1*	<1	Unknown	Unknown	No
Hereditary breast ovarian cancer syndrome (HBOC)	*BRCA2*	5	3.5-10	3.5	Yes
Li–Fraumeni syndrome	TP53	<1	<5	<5	No
Familial adenomatous polyposis	APC	<1	<5	<5	No
Cystic fibrosis	CFTR	0	<5	<5	Yes
Ataxia telangiectasia	*ATM*	<1	Unknown	Unknown	Yes
Possible FPC	*PALB2*	<1	Unknown	Unknown	Individual basis

7.1.6 The International Cancer of the Pancreas Consortium

The International Cancer of the Pancreas Screening (CAPS*)* consortium met in 2011 and defined potentially relevant findings in screening as early PDAC (T1 N0 M0 R0), grade 3 PanIN, and high grade BD or MD IPMN.(223) To quantify the number of positive findings identified in the various pancreatic cancer screening programs running internationally, 21 published screening reports were reviewed (226, 308-327) with a total of 30 pancreatic cancers and two pancreatic neuroendocrine tumours were reported. The most common positive findings were cystic lesions, including six MD-IPMNs and 60 BD-IPMNs. Of the 1,780 individuals who were screened, 131 underwent surgical resection with the indication for surgery being 'the identification of a positive finding during screening'. The published outcomes of various screening programs from groups including Johns Hopkins Hospital (JHH), the University of Washington (UW), the Memorial Sloan-Kettering Centre (MSKC), the German National Case Collection for Familial Pancreatic Carcinoma (FaPaCa), the Spanish National Hereditary Pancreatic Cancer Registry (PanGen-FAM), the Danish national screening program and the Swedish screening program are summarized in Table 24.

Table 24. A summary of the published results of screening programmes for FPC and IARs for PDAC.

Study	Number included	Programme base	Duration of Follow-up / study period	PDAC	MD IPMN	Surgery	Other findings
Brentnall et al (308), 1999 #	14	UW (USA)	-	0	0	7	dysplasia
Kimmey et al (309), 2002 #	46	UW (USA)	5 years	0	0	12	12 dysplasia
Canto et al (310), 2004 #	38	JHH (USA)	1998-2001	1		7	1 IPMN 2 SCA 3 PanIN 1-2 1 PanIN 3
Canto et al (311), 2006 #	227 (78 FPC/PJS, 149 controls)	JHH (USA)	2001-2004	0	0	7	7 IPMN (1 progressed to advanced PDAC during FU) 1 PanIN
Langer et al (312), 2009 #	76	FaPaCa (Germany)	1999-2007	0	0	6	1 BD-IPMN 3 SCA 1 PanIN 2 1 PanIN 1

Poley et al (313), 2009	44	Netherlands	2005-2007	3^	0	10	7 BD-IPMN
Verna et al (314), 2010	51	Columbia (USA)	2005-2008	2	0	5	5 BD-IPMN 7 other cystic lesions
Ludwig et al (315), 2011	109	MSKCC (USA)	2002-2009	1	1*	6	2 BD-IPMN 1 SCA 1 PanIN3 1 PanIN2
Schneider et al (226), 2011	72	FaPaCa (Germany)	1999-2009	1	0	9	3 SCA 1 PanIN 3 2 IPMN 1 PanIN1/2
Vasen et al (316), 2011	79 FAMMM only	Netherlands	2000-2010 (median 4, range 0-10 years)	7 (3 ^)	0	5	9 other cystic lesions
Zubarik et al (317), 2011	27	UVM (USA)	2006-2009	1	0	3	1 NET 1 PanIN1
Al-Sukhni et al (318), 2012	262	Canada	2003-2011 (av 4.2 years)	3		4	15 BD-IPMN 65 other cystic lesions 1 pNET
Canto et al (319), 2012 #	216	CAPS (USA)	Median 28.8 months (range, 14– 47.2 mo).	0	2	5	82 Cystic lesions 5 resected = 2 MD IPMN and 3 BD-IPMN) 3 pNET

Potjer et al (320), 2013 #	125 FPC	FaPaCa (Germany)	Median FU 34 months	1	1	11	51 other cystic lesions Including: 5 BD-IPMN with 4 PanIN 2-3, 1 PanIN 1 3 SCA 3 PanIN 1 only
Sud et al (321), 2014	30 (Inc PJS and BRCA2)	USA	2008-2011	2 ^		3	1 IPMN (LGD)
Mocci et al (322), 2015	41	PanGen-Fam (Spain)	2 years	0	0	1	1 pNET 1 PanIN 3
Joergen-sen et al (324), 2016	40 FPC (31 HP)	Danish national screening program (Netherlands)	2006-2014	2	-	2	
Harinck et al (325), 2016	139	Dutch research group on PC (Netherlands)	12 months	1		2 (PDAC, multifocal PanIN2)	9 cystic lesions 1 PanIN2
Del Chiaro et al (323), 2015	40	KUH (Sweden)	2010-2013 (mean 12.9 months)	3 (2^)	2 ^	5 (3 PDAC, 2 IPMN)	9 BD-IPMN 3 mix type 1 IPMN (1 with PDAC)
Bartsch et al (326), 2016	253	FaPaCa, The Leiden and Madrid registry, (Germany)	2002-2015 Median 28 (1–152) months	2	0	21	1 BD-IPMN with HGD 3 SCA 1 pNET 5 PanIN 2-3 6 PanIN 2 with BD IPMN
Total	1780			30	6	131	23 PanIN2-3 2 pNET

7.1.7 EUROPAC FPC registry and secondary screening study

EUROPAC recruits families with either Hereditary Pancreatitis (HP) or Familial Pancreatic Cancer (FPC) and offers cancer screening on a research basis. The HP arm of EUROPAC has been previously discussed, therefore this chapter will only address work relating to the FPC EUROPAC cohort.

In some of the EUROPAC FPC families a known causative mutation has been identified (mutations in *BRCA2*, *CDKN2a, STK11* or mismatch repair genes). These are associated with autosomal dominant predisposition that can be confirmed by segregation of the mutation with cancer cases. However, in most of our families DNA sequencing has not been possible in enough cases to confirm segregation, so we cannot rule out low penetrance in some of our families despite the presence of known causative mutations in screened individuals (e.g., a BRCA2 mutation cannot be guaranteed to equate to very high risk because the individual may have a protective genetic background not seen in high-risk families with BRCA2). In the majority of FPC families no known causative mutation has been identified and DNA sequencing cannot be used to distinguish families with genuine autosomal dominant predisposition from families where the cancer cluster occurs by chance or due to a polygenic predisposition. Estimates of relative risk vary between 6 and 120 fold depending on the nature of family selection,(306, 328) the 120 fold level being most consistent with autosomal dominant predisposition and lower values perhaps indicating a higher proportion of random clusters, which will not give adequate elevated prospective risk for screening.

This chapter describes the results from EUROPAC's pilot screening study. These data were collated, quality assured and analysed during my period serving as the EUROPAC clinical fellow. In total, one PDAC case and a MD-IPMN were identified along with two low grade neuroendocrine tumours, but the most frequent findings were cystic lesions, the most common of which were BD-IPMNs. If BD-IPMNs can genuinely be considered a positive finding in screening, then it would be logical to assume that they should be more frequently encountered in individuals with the highest risk of pancreatic cancer

because of autosomal dominant predisposition. We have assessed if familial risk (as opposed to risk due to other factors such as age or smoking) correlates with a higher incidence of IPMNs in our FPC kindreds.

7.2 Aims and hypothesis

The purpose of this study was to evaluate the relationship between screening results and familial predisposition. Familial risk of PDAC correlates with a higher incidence of IPMNs in our **FPC** kindreds.

7.3 Materials and Methods

7.3.1 Patients and ethics

EUROPAC is a patient led registry (ethical approvals MREC 03/8/069 and 07/H1211/96). Screening inclusion depended on at least two first-degree relatives with confirmed PDAC or a high-risk mutation (in *BRCA2*, *CDKN2a*, *MLH1*, or *STK11*). All had to be aged over 40 years or 10 years younger than the youngest affected first degree relative.

Epidemiological data were collected via questionnaires supported by clinical consultations and stored on a database (Progeny version 8.01) in accordance with the UK Data Protection Act (1998). Matched DNA was kept under the care of the Merseyside and Cheshire Genetics Service. Recruitment for screening was patient led, with approximately 40% uptake.

7.3.2 Screening protocol

The EUROPAC FPC screening protocol employed during this study period is summarised in Figure 24. Baseline serum glucose and Ca19-9 were performed alongside imaging (both pancreas protocol computed tomography and endoscopic ultrasound of the pancreas). Following this there is a three yearly screening cycle. Consenting individuals had collection of duodenal juice, with secretin stimulation, and molecular analysis (previously this was done by cannulation of the pancreatic duct). If the juice contained no cancer

associated genetic abnormalities, participants entered a three yearly screening cycle, with staggered EUS and/or Magnetic Resonance Imaging. In patients without juice collection or with cancer associated mutations in their juice, there was an annual pathway consisting of repeat blood testing and EUS. Any abnormalities identified in imaging or molecular tests are discussed at the supra-regional pancreatic multi-disciplinary team (MDT) meeting. The MDT may recommend further clinical investigations, surgery or that the participant undergoes annual surveillance or regular clinical review and/or follow up.

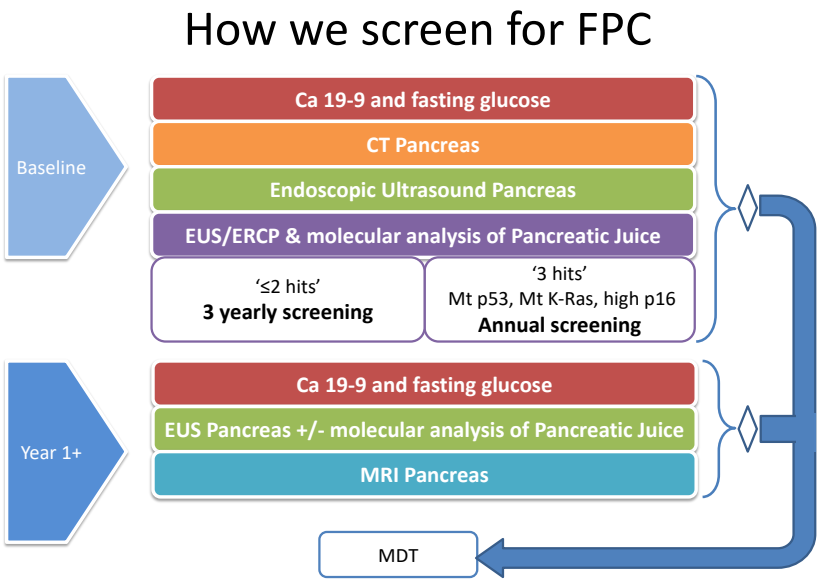


Figure 24. EUROPAC screening protocol as of Oct 2016

The 2016 protocol consisted of three yearly screening cycle following a baseline assessment consisting of CT, EUS and blood tests. There is EUS imaging at the end of each cycle, followed by collection of pancreatic juice and molecular analysis the year after. In patients who do not have juice collection or who had a cancer associated mutation in their duodenal juice, there is an annual pathway consisting of repeat blood testing and EUS/MRI.

7.3.2.1 Molecular analysis of duodenal juice

Extracted DNA from juice was quantified by real-time PCR for a specific genomic DNA sequence (*KRAS*). The methodology for molecular analysis has been described:(329) a yeast functional assay was used to identify p53 mutations, Amplification Refractory Mutation System was employed for analysis of *KRAS* mutations and a real-time methylation specific PCR assay for *CDKN2a* promoter methylation. For later analysis deep sequencing of *Tp53* was carried out on juice samples using an Ion Torrent Personal Genome Machine. Libraries were constructed as described in manufacturers protocols(330) following dilution of DNA to 10 genomes per reaction.

7.3.3 Risk calculation

The purpose of this study was to evaluate the relationship between screening results and familial predisposition, risk was therefore only evaluated in terms of family structure. Other factors, such as age and smoking that increase risk of cancer but may also increase risk of cystic lesions, were not included in this analysis, but have been included in more complex model development and analyses completed and presented in the thesis of my predecessor, Mr C Grocock.

To simplify the analysis to answer our specific aim, we developed the novel concept of *family index (FI)* for the purposes of this work, whereby the number of cases of PDAC in each family was taken as the numerator and the square root of the number of at-risk individuals as the denominator. To allow for no individuals of 40 or above, one was added to the number at risk.

$$FI = \frac{Number\ of\ affected\ individuals}{\sqrt{[(Number\ of\ individuals\ in\ kindred \geq 40\ years)+(1)]}}$$

Mendelian principles were used to calculate the chance of inheriting a high-risk allele. For example, any first degree relative of a pancreatic cancer case has a 50% chance of being a mutation carrier, reducing to 25% in a nephew or niece. If a potential causative mutation was identified the chance was considered as 100%. *FI* was multiplied by percentage chance of inheritance to

give an arbitrary score for risk (e.g. an individual in a family with *FI* = 0.5 and a 50% chance of being a carrier has a risk score of 25.

The arbitrary risk score calculated was compared in prospective cancer cases on the registry to a division of families based simply on number of cases and number of generations affected.

This concept has been refined further and the current correct terminology used by EUROPAC is Family risk (FR). FI x 100 = FR.

7.3.4 Statistical analysis

Continuous variables are presented as median with Inter quartile range (IQR). Risk groups were created pragmatically based on tertiles of the whole screened population, giving low, medium and high risk. The numbers of each finding in each tertile were compared using Pearson Chi Square or Fisher's exact testing as appropriate.

7.4 Results

The demographics of all individuals consented and recruited to the FPC EUROPAC registry are described in table 25. Polygenic conditions can lead to high risk in a single generation, with autosomal dominance more likely to give cases in multiple generations. In table 26 the families are therefore split according to number of pancreatic cancer cases and generations affected.

In table 26 all families that would have included individuals (registered or unregistered relatives) eligible for screening in the year 2000 are shown, broken down by family type as in table 25. The number of prospective cancers (i.e. cancers occurring after 2000 and before October 2016) is given, confirming the high risk of cancer in this cohort.

Table 25. Demographics of EUROPAC registered individuals and the cancer cases in their families in October 2016 for comparison with the screened cohort

Family Type	Total Registered Individuals	Total Kindreds	Total Number of PDAC in Kindreds	Age (Current) *Range/Mean*	Gender	Smoking	Number of Individuals Screened
FPC ≥3 cases multi generations	308	145	504	24-99 58.18	F=195 M=113	Yes=41 No=145 Ex=94 Unknown=28	84 individuals *60 families*
FPC 2 cases 2 generations	438	281	562	24-91 55.54	F=275 M=163	Yes=43 No=211 Ex=135 Unknown=49	128 individuals *105 families*
FPC ≥3 cases 1 generation	44	25	80	32-85 57.05	F=27 M=17	Yes=7 No=13 Ex=14 Unknown=10	6 individuals *6 families*
FPC 2 cases 1 generation	190	110	220	25-91 56.85	F=130 M=60	Yes=26 No=97 Ex=56 Unknown=11	47 individuals *39 families*
BRCA2	81	54	39	28-84 57.02	F=58 M=23	Yes=4 No=37 Ex=32 Unknown=8	25 individuals *22 families*
FAMMM	27	14	24	34-67 49.1	F=19 M=8	Yes=7 No=15 Ex=4 Unknown=1	9 individuals *8 families*
PJS	7	6	2	19-69 43.57	F=2 M=5	Yes=1 No=3 Ex=2 Unknown=1	4 individuals *4 families*

HNPCC	20	15	21	41-76 60.05	F=12 M=8	Yes=0 No=9 Ex=7 Unknown=4	8 individuals *5 families*
Other	84	66	83	30-92 58.86	F=55 M=29	Yes=8 No=43 Ex=22 Unknown=11	10 individuals *9 families*
Totals	1199	716	1535	N/A	F= 773 M=426	Yes= 137 No= 573 Ex= 366 Unknown= 123	321 individuals 258 *families*

[*] Four individuals were recruited for screening from a family with a CDKN2a mutation but were later found to not be carriers

[ɣ] Four individuals with MLH1 mutations and 4 defined on family history alone

[Ψ] Families with cancer syndromes (none with known causative mutation

Table 26. Individuals in EUROPAC Kindreds followed from 2000-2016 showing prospective cancers to demonstrate a high-risk population

	All individuals				Just pancreatic cancer cases				New cancer cases 2000-2016
	Total Individuals (Kindreds)	Age (median & IQR)	Gender M=Male F=Female	Smoking*	Total Cancer Events	Age (median & IQR)	Gender M=Male F=Female	Smoking*	
Multi-generation ≥ 3 cases	1044 (44)	49 (29-64) N=747	M=532 F=512	Yes=32 No=61 Ex=33 Child=93 Unknown =825	158	61 (54-68) N=140	M=83 F=75	Yes=12 No=13 Ex=4 Child=0 Unknown=129	23
Two generations 2 cases	1942 (109)	44 (24-62) N=1356	M=961 F=981	Yes=50 No=96 Ex=60 Child=224 Unknown =1512	218	64 (56-72) N=204	M=96 F=122	Yes=16 No=16 Ex=5 Child=0 Unknown=181	30
Single generation ≥3 cases	553 (15)	53 (33-69) N=248	M=277 F=276	Yes=3 No=5 Ex=5 Child=21 Unknown =519	52	65 (58-71) N=47	M=26 F=26	Yes=1 No=0 Ex=0 Child=0 Unknown=51	**5**
Single generation Two cases	1201 (57)	51 (34-67) N=748	M=574 F=627	Yes=25 No=42 Ex=32 Child=68 Unknown =1034	115	64 (56-72) N=104	M=52 F=63	Yes=7 No=2 Ex=4 Child=0 Unknown=102	20

Table 27. Prospective cancer cases between 2000 and 2016 showing the range of familial risk

	New Cancer Cases (2000-2016)				Median FI at diagnosis (IQR)	Median Risk score at diagnosis (IQR)
	Total Cancer Events	Age (median & IQR)	Gender M=Male F=Female	Smoking*		
Multi-generation ≥ 3 cases	23	73 (60,80) N=23	M=11 F=12	Yes=13 No=6 Ex=3 Unknown=1	1.0(0.9,1.3)	50.0(43.3,75.3)
Two generations 2 cases	30	68 (61,74) N=30	M=20 F=10	Yes=23 No=1 Ex=3 Unknown=3	0.7(0.6,0.8)	33.3(28.9,41.8)
Single generation ≥3 cases	5	68 (57,80) N=5	M=2 F=3	Yes=3 No=1 Ex=1 Unknown=0	1.0(0.8,1.4)	48.5(41.0,69.7)
Single generation Two cases	20	68 (57,80) N=20	M=8 F=12	Yes=13 No=4 Ex=3 Unknown=0	0.7(0.6,0.8)	37.8(32.0,40.8)
Total	78	68 (60,75) N=78	M=41 F=37	Yes=52 No=12 Ex=10 Unknown=4	0.8(0.6,1.0)	40.0(31.6,50.0)

The prospective cancer cases are described in table 27. There was a trend for the prospective cases to have slightly older age of cancer onset than the historical cases and this trend was seen in all categories of family. The median arbitrary risk score for all prospective cancer cases was 40, with the highest values in families with more cases of cancer (e.g. 3 cases in more than one generation having a median risk score of 50). Seventy-five percent of prospective pancreatic cancer cases had an arbitrary risk score at diagnosis of greater than 31.

Figure 24 shows the EUROPAC protocol for screening members of FPC kindreds. As of October 2016, there were 3031 individuals who would be eligible for secondary screening; however, only 791 of these were registered with the EUROPAC registry. These 791 patients were informed of their eligibility and from this point onwards, uptake of screening was entirely patient led (the team did not approach patients beyond informing them of their eligibility for screening). After confirm eligibility for screening, 321 individuals from FPC kindreds volunteered to participate in the screening research programme. It is important to highlight that this is only 10% of all the eligible individuals on the disease (321/3031, 10.6%). 123 participants had completed more than 2 screening cycles, 46 had completed two cycles, 71 one cycle and 11 had a finding on baseline screening investigations; giving a total of 786 screening cycles completed. In addition, there were 70 individuals who had been recruited and undergone baseline screening but with less than one year of follow up and with no abnormal findings. The median screening follow-up was 2 years (IQR 0-5) and the median number of screening investigations per participant was 4 (IQR 2-6).

The findings from screening are summarised in table 28 and Figure 25. The most common findings were cystic lesions: 41 cystic lesions were identified, of which 1 was a main-duct IPMN and 22 were branch-duct IPMN. The other cystic lesions were too small for definitive radiological classification; although these may have been very small branch-duct IPMN, it does rule out larger lesions such as mucinous cystic neoplasms. Two Pancreatic Neuroendocrine

Tumours (pNETs) were discovered, both were resected and were found to be well differentiated. One PDAC was identified. In addition, a Gastro-Intestinal Stromal Tumour (GIST) was discovered in the stomach of one patient. Three pancreatic resections were performed: for both pNETs and for the MD-IPMN. Histological examination of the specimen from the MD-IPMN revealed low grade dysplasia of main lesion and also revealed an incidental branch-duct IPMN with low grade dysplasia. The one PDAC case identified was unfortunately advanced at the time of diagnosis and was therefore inoperable. Within the screening cohort, there were four deaths from all causes: one being the advanced PDAC, two from extra-pancreatic malignancy, and one cardiac related death.

Table 28. Screening events stratified by risk group.

	Low Risk	**Medium Risk**	**High Risk**
PDAC	0	0	1
pNET	1	1	0
MD-IPMN	0	1	0
BD-IPMN	6	10	6
Other Cystic Lesions	5	6	7
Total findings (total follow-up years)	12 (260 screening follow up years)	18 (289 screening follow up years)	14 (239 screening follow up years)
Finding/Follow-up year	0.05	0.06	0.06

The most frequently used screening modality was EUS. CT was only performed as part of baseline investigation, unless clinically indicated due to positive findings in other modalities. MRI was only routinely introduced into the screening protocol from 2014.

Of 35 screened individuals with a known causative mutation only two had a significant finding on screening; one out of 22 with a *BRCA2* mutation had a 10mm BD-IPMN, that regressed during clinical follow up, one out of 5

individuals with a *CDKN2a* mutation had an 11mm BD-IPMN which has remained stable after 36 months of follow-up.

Only 48 participants consented for ERCPs with molecular analysis of pancreatic juice and only four patients had positive molecular test results in at least two analyses. This is too small a group to make any significant conclusions. Two of these patients had cystic lesions that were too small for further characterisation. Both of the remaining participants with two positive tests had EUS findings consistent with minimal change chronic pancreatitis (although neither had symptoms or diagnosis of pancreatitis and the imaging abnormalities resolved). The single PDAC case and one of the pNETs did not undergo pancreatic juice molecular analysis. The other pNET had undergone 2 separate pancreatic juice collections. The first gave wild type *KRAS* and normal levels of *CDKN2a* promoter methylation (0.01%), *Tp53* analysis failed. The second test gave wild type *KRAS* and *Tp53*, with *CDKN2a* analysis failing. The MD-IPMN case did not undergo pancreatic juice analysis in the screening cycle where the lesion was identified, in a previous analysis they had wild type *KRAS* and *Tp53* with normal *CDKN2a* promoter methylation (0.018%).

In Figure 25 each screening event is shown with the outcome colour coded, red for the cancer, pink for the pNETs, amber for the main-duct IPMN, green for no significant finding etc. The participants are ranked according to the risk score of the individual at the time of screening estimated as above. Four individuals were included for screening because of family history but were found not to have the disease mutation (a *CDKN2a* mutation) identified subsequently in this family, they therefore were classified as having a zero elevated risk. The PDAC case was identified in an individual who at the time of screening had 5 cases of PDAC in the family and 17 individuals in the family tree over the age of 40. The FI was therefore 1.179. There was a 50% chance of the individual being a carrier so the risk score was 58.95. This put the individual's familial risk in the top 10% of risk scores in the screened population. Splitting the screening participants evenly into three groups (low,

medium and high) as shown in Figure 25 indicated no correlation between risk and incidence for branch-duct IPMN (χ^2 = 0.937, P= 0.632).

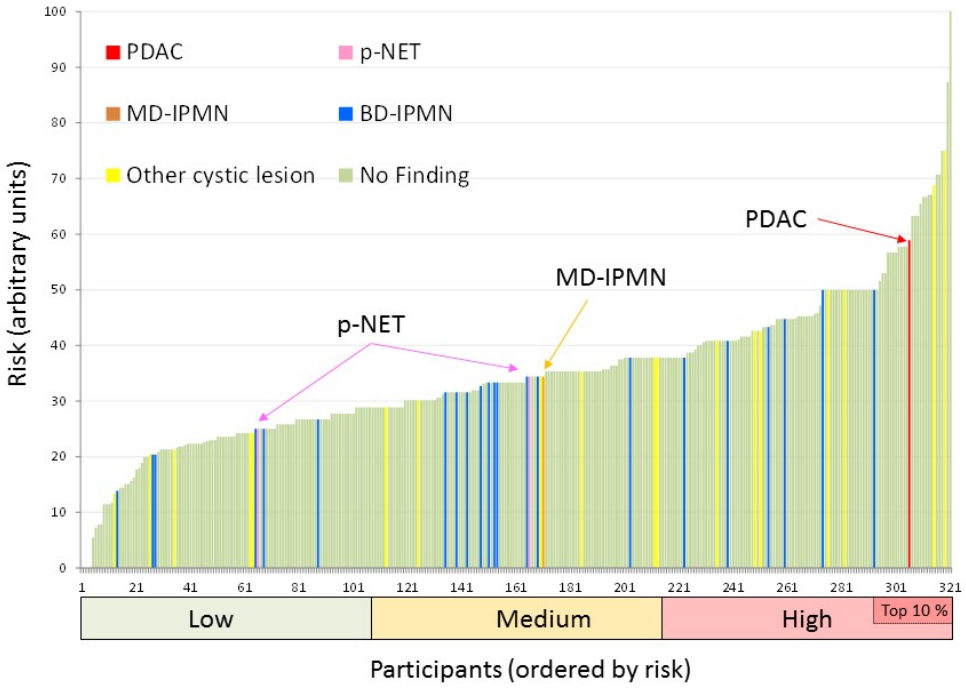


Figure 25. Screening results stratified by risk that an individual carries a high penetrance mutation predisposing to pancreatic cancer.

Risk was estimated based on number of cases of cancer, number of at-risk individuals in each family and the chance that a participant was carrying an autosomal dominant determinant of cancer predisposition. Each individual is then represented by a coloured bar indicating the outcome of screening (as shown in the key). Positive events are indicated with arrows and the tertiles of risk (along with the 10% upper risk group) are indicated by boxes below the x-axis.

7.5 Discussion

Screening was carried out under the assumption of autosomal dominant predisposition for PDAC. The probability of a cluster of PDAC without such

predisposition will increase with the number of at-risk individuals in a kindred and will reduce with the number of pancreatic cancer cases. Risk for an individual will depend on their age, exposure to environmental risk factors and lifestyle, but none of these factors, alone or in combination, would merit inclusion of an individual in a screening programme, nor would they influence the prospective risk of other family members. A screening finding must therefore be judged according to the genetic risk of an individual. The one case of PDAC occurred within the top 10% of familial risk and the one case of MD-IPMN was identified in a medium risk family. Twenty-two branch-duct IPMN were identified with equal probability in individuals of all familial risk categories.

Five year follow up of 367 individuals from the population-based Study of Health in Pomerania (SHIP) identified 48 participants who developed cystic lesions (12.9%). Although the SHIP study is not directly comparable with the prospective screening described here, it is notable that we identified a total of 41 cystic lesions in our population of 321 participants (12.8%), so our data is entirely consistent with the expected discovery of cystic lesions in the general population (331). Age is a risk factor both for the development of pancreatic cancer and IPMN (as shown in the SHIP analysis), we deliberately did not include age in our risk model, as the question was whether genetic predisposition increased the risk of cystic lesions. Our hypothesis was that the cystic lesions were intermediates in a genetic predisposition for pancreatic cancer and could therefore be taken as a positive result in a cancer screen. The cystic lesions within the EUROPAC screening cohort were more common in older participants, but this was true even for the low-risk group, although very few prospective cancers occur in this group of patients and presumably many of the individuals in this group were at no greater risk of pancreatic cancer than any other individual of a similar age. The BD-IPMNs were also no larger or more likely to progress in the high-risk group than in the low-risk group.

However, although an individual with a BD-IPMN may be at greater risk of cancer, our data suggests individuals with a higher inherited risk of PDAC are

not at a higher risk of developing BD-IPMN. Thus, the EUROPAC study does not support the inclusion of non-malignant pancreatic cystic lesions, including branch-duct IPMNs, as positive findings on screening individuals from FPC families. In this EUROPAC may appear to be in opposition to other screening groups who do consider cystic lesions as intermediates in the development of pancreatic cancer in patients with a familial predisposition. However, taking the CAPS data presented in 2022 as an example neither our data or our conclusions are substantially different, the screening yield of IPMN and other cystic lesions was equivalent and although 10 patients who were resected with IPMN did have high grade dysplasia, none of the resected PDAC were histologically associated with an IPMN. The CAPS consortium advocate surveillance of IPMN identified through screening and so do EUROPAC, the only difference being EUROPAC's explicit statement that the presence of the IPMN is its own justification for that surveillance and should not be convoluted with the separate (but very real) risk associated with a family history (332).

The screening results presented here are consistent with the outcomes described by other groups, with discovery of cystic lesions far outweighing identification of PDAC (226, 308-318, 320-327, 333). The poor return of screening programmes can be explained by inclusion of too many low-risk individuals in the screening cohorts. Any individual's chance of being at high-risk will be the same as the chance of carrying a predisposing mutation (e.g. 50% for a first degree relative). The actual risk will be lower because superimposed is the chance that the family may just represent a random cluster of cases. This means that most individuals undergoing screening for PDAC on the sole basis of family history of the disease have no elevated risk. No elevated risk of PDAC means no elevated risk of precursor lesions.

In 2007 Wang et al developed the PancPro Mendelian model to identify high-risk individuals within FPC kindreds (288). In our report we used a much simpler (pragmatic) risk score based on the number of cases of pancreatic cancer in the family, which is the most widely recognised measure of familial risk(328), with the added advantage of stratifying risk within groups of families

with equal numbers of pancreatic cancer cases. This arbitrary risk score although inferior to PancPro in accuracy for quantifying PDAC risk, has the advantage for our purpose that it avoids factors that would apply to sporadic pancreatic cancer and cystic lesions, such as age and smoking. Independence of such risk factors was essential in showing that the familial predisposition for cancer was largely (or entirely) independent of risk of developing BD-IPMN. The prospective reporting of new cases of PDAC in individuals at higher familial risk than those being screened indicates the need for a strategy to encourage more high-risk individuals to participate in screening. By restricting screening using PancPro (or equivalent) it should be possible to focus resources on encouraging higher risk individuals to participate.

The results from this work were highly influential during the evolution of the EUROPAC screening protocols. The concept of *family index* introduced here was combined with additional factors to develop a risk stratification or Family Risk (FR) score. As demonstrated in Figure 26, the EUROPAC FPC screening protocol now utilises an individual's FR score to classify them as low or high risk which ultimately will influence their eligibility for both the registry and enrolment onto the screening programme with the aim to further enrich the screening population and increase the yield of PDAC early detection.

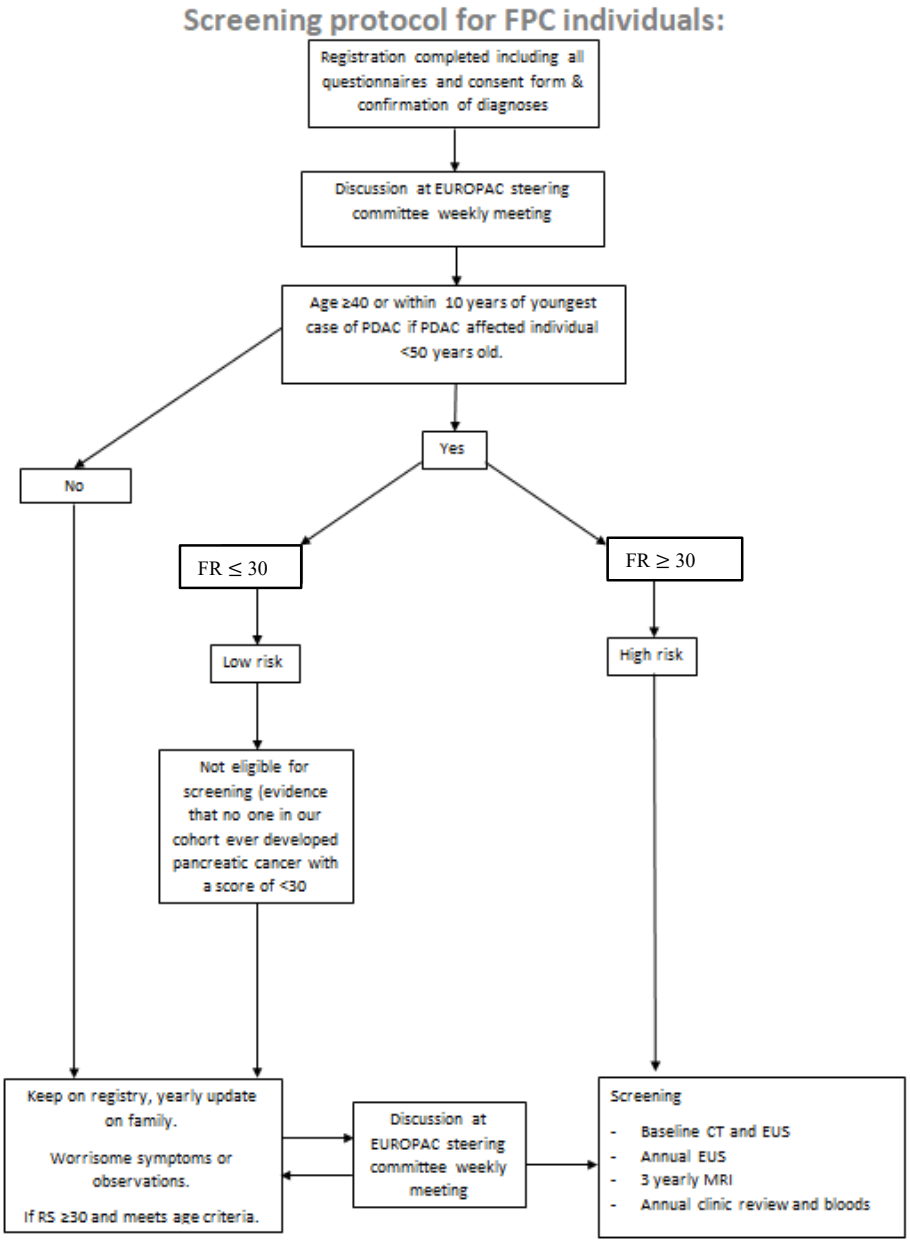


Figure 26. The 2021 updated EUROAPC FPC screening protocol, taking FR into account.

7.6 Conclusion

Stratification of the FPC screening cohort by family history made no difference to yield of cystic lesions within FPC kindreds. An increased frequency of IPMN in higher-risk individuals could have supported the conclusion that FPC predisposes to the development of IPMN which in turn predisposes to PDAC. This was not the case, therefore the hypothesis that IPMN are an intermediate stage in the development of PDAC within FPC kindreds is rejected. IPMN identified during screening should on this basis be treated in the same way as IPMN discovered incidentally in the general population (according to the appropriate guidelines). A desirable feature of risk stratification is that it is unlikely to increase the yield of branch-duct IPMN.

Chapter 8: Discussion

8.1 Overview

The clinical entities discussed in this thesis including early CP, CP, precursor lesions, and PDAC are indeed complex disorders of the pancreas. There are multiple mechanisms and pathological pathways that may disrupt normal pancreatic function or response to stress or injury which contribute to the development of these diseases. All these disorders share a common theme, an early disease phase. This presents an opportunity for identification of those at higher risk of disease, earlier disease detection, potential screening, and initiation of early therapies which may slow down, halt or even reverse disease progression. Given the complex and intimate nature of the relationships between these diseases' early treatment of any one of them may also prevent the development of pancreatic cancer.

8.2 CP and early CP

General adoption of the new mechanistic definition of CP (10) has led to a paradigm shift in approach to understanding chronic pancreatitis. Its introduction heralded a move away from defining the disease purely based on the classical end stage histopathological features and symptoms, with a new focus on the underlying disease mechanisms and risk factors. The definition has provided a framework for defining the complex aetiologies that drive progression of the disease through this general pathway and for identifying disorders in the differential diagnosis that have overlapping features but different aetiologies and outcomes.

CP is a disorder possessing many features that overlap with other conditions that are not related to the pancreas. The potential benefits of early diagnosis of CP have been well described however, until there is clarity regarding the definition and diagnostic criteria for early CP, one must balance these potential

benefits against the risks of an incorrect diagnosis of CP. This may lead to stigmatisation of the individual and even more concerning, may set a patient off on a management path of opiate medication, ever increasingly invasive investigations, interventional endoscopy and potentially even cumulate in life altering surgery such as TPIAT.

8.2.1 The prospective follow up of early CP

The approach to CP and early CP in this thesis was strongly influenced and inspired by the mechanistic definition of CP and the conceptual model of CP by Whitcomb. According to this conceptual model (10), CP will develop in those "at risk" (stage A) if the pancreas is exposed to injury/stress. Disease onset is heralded by AP/RAP (stage B), which can result in a chronic inflammatory response progressing to early CP (stage C). A fundamental aspect of this model is the potential for the resolution of early CP. Meaning there is a reversible element to the pathological process. Repeated or persistent exposure to stressors or injury causes pancreatic dysfunction relating to immune response, acinar cells, endocrine function and pancreatic architecture resulting in established and irreversible CP. Disease can progress to the final stage where the most serious sequalae are seen including severe fibrosis, pancreatic failure, vascular involvement, intractable pain and pancreatic cancer development. Following a full clinical review of The Liverpool CP cohort, patients were attempted to be classified according to the five disease stages as proposed by Whitcomb. Whilst this is not a true validation of the model, it does show that clinical prospective validation could be feasible and that the model can be a useful tool to classify challenging patients. We feel this model resembles our clinical experiences and CP population seen in Liverpool.

Data presented in this thesis support the fundamental principle of the conceptual model that there is potential for the regression/resolution of early disease. As far as we are aware, data relating to resolution of MCEUS changes presented in this thesis are the first description of resolution of MCEUS changes published in the literature (143). Five patients with initial MCEUS changes showed complete resolution of EUS findings on clinical follow up after

removal of their susceptibility factors (alcohol and tobacco exposure). Disease progression was demonstrated in 12 out of 40 (30%) patients who did not have definite evidence of CP on imaging findings over a 30-month period. Over two thirds of these patients continued to drink excessive amounts of alcohol and 75% continued to smoke.

8.2.2 Clinical implications

Data presented in this thesis demonstrates firstly that there are overlapping disorders with very similar clinical pictures to CP that on long term follow up and retrospective review of the patients' record are in fact not CP. We have highlighted the diagnostic challenges surrounding these patients and would subsequently advise that CP is not formally diagnosed outside of a tertiary referral pancreatic centre with expertise in managing CP. We have outlined guidance on the timings of imaging investigations for CP following an episode of AP and have advised that caution is taken when interpreting EUS features of early CP within 12 months on an acute pancreatitis episode. We also advise that individuals with MCEUS changes or suspicions of early CP are followed up clinically for at least a period of 30 months before a formal diagnosis of CP is made. In the meantime, any patients under surveillance or follow up should be given clear lifestyle counselling to encourage disease modifying behaviours including exclusion of all alcohol and tobacco consumption which may slow or halt the progression to definite CP if they do indeed have early CP.

8.2.3 Limitations

8.2.3.1 High rate of advanced disease

The CP population reviewed by the pancreatic services at The Royal Liverpool University Hospital may not be entirely representative of other geographical locations and therefore some of the findings presented in this thesis may not be directly applicable, or transferable to other patient populations.

A large proportion of advanced and end staged CP disease was observed within the Liverpool CP cohort which appears disproportionate to other comparable regional and inter/national populations. High levels of pancreatic

calcifications on cross sectional imaging were noted. Calcification is typically associated with CP caused by alcohol excess and/or genetic mutations. We also noted that nearly half of the patients in the cohort had undergone pancreatic surgery at some point during their management.

The exact reasons for this observed difference are unclear. Liverpool and the surrounding areas have some of the highest levels of socio-economic depravation within the UK and this is commonly associated with increased rates of substance abuse including alcohol dependence and tobacco smoking.

Pancreatic services at The Royal Liverpool University Hospital are a supra-tertiary specialist referral centre with an internationally renowned reputation for high quality pancreatic research which includes an interest in inherited diseases of the pancreas. It is therefore logical to assume that there would be a greater number of patients with genetic disorders, known mutations, and strong family histories referred to our team. This would bias the distribution of CP aetiology in our cohort towards the more complex and less common.

Due to the large number of patients (85%) who already had established CP (which was often already advanced at first presentation to the pancreatic services), the number of patients with the potential for observed disease progression during follow up was small.

8.2.3.2 The limitations in diagnosis of early CP

The two biggest limitations to diagnosis early CP are;

1) the lack of a widely accepted definition of this disease stage.
2) The lack of a gold standard diagnostic test and the lack of an accepted number of diagnostic criterions required for early CP diagnosis (163).

Diagnosing early CP is one of the greatest challenges faced by pancreatologists. A working group of international pancreas experts were unable to reach consensus on the definition of early CP, but they did agree that early CP can be diagnosed in theory based on the presence of patient risk

factors, low probability for other disorders with overlapping features, appropriate clinical context and supportive biomarkers (163).

In addition to the lack of a definition of the condition, commonly used diagnostic techniques do not have the required sensitivity or specificity to diagnose early disease. Histopathological features are unreliable in early CP as there is overlap in appearances of the pancreata of individuals without clinically evident pancreatic disease especially in cases of advancing age, obesity, diabetes mellitus, alcohol excess and smoking (334-339).

EUS, MRI and secretin stimulated MRCP remain the commonest modalities used to detect early pancreatic morphological changes available at present (57, 91, 163, 340). EUS is thought to be the most sensitive. The classically reported EUS related findings such as hyperechoic foci and strands, parenchymal lobularity and hyperechoic ductal wall are all understood to be signs or surrogates of pancreatic fibrosis (337, 341). These findings are sensitive but not specific to CP (97, 340, 342).

The number and types of EUS based diagnostic criteria continue to be a source of controversy. There are several published diagnostic criteria for early CP which are based on features identified during EUS including the standard criteria (94), the Rosemont criteria (95) and the Japanese criteria (96). None have been validated and none have been accepted internationally. This is because there is no agreement on the threshold number of EUS features required to make a diagnosis of early CP, although generally as the threshold number of criteria increases the specificity for a diagnosis of CP increases, but the sensitivity decreases (343).

Diagnostic accuracy of EUS is further hampered by interpreter bias based on a patients clinical history and known risk factors (which in theory would make a diagnosis of CP more or less likely and thus may influence the interpretation), suboptimal interobserver agreement, and potential for intraobserver variability (344, 345).

There are some clinicians that would disagree, however currently I do not believe that early CP can be diagnosed by abdominal imaging techniques alone. There is a need for future prospective studies to determine the accuracy of early CP diagnosis in groups with overlapping imaging features as described above.

8.2.4 Future work

8.2.4.1 Clinical validation of the mechanistic definitions

The new mechanistic definition of CP(10), the progressive pathogenesis model and the SAPE phenomenon (66) provide a critically needed frameworks for designing new intervention studies for the management of CP. Neither the definition nor the associated models have been prospectively validated, however.

8.2.4.2 Revision of current terminology

There needs to be more stringent application of the systems used for diagnosing chronic pancreatitis with revision of the current terminology. At present terms such as ‘indeterminate’, ‘suggestive’, ‘possible’, ‘early’, mild CP, minimal change CP, and non-calcific CP, are all used interchangeable to refer to “early” chronic pancreatitis. This is not only confusing for patients, clinicians, and researchers alike but prevents standardised classifications to allow global collaborative research and exchanging of data.

Without a definition or diagnostic criteria for early CP it has not been possible to conduct high quality prospective studies.

8.2.4.3 Biomarkers for disease progression

During the International CP guideline process, international experts agreed that despite histopathological features of CP being well defined and internationally accepted, histopathological assessment is not the gold standard for the diagnosis of CP. Especially as there are no accepted scoring systems available to assess factors that reflect disease progression and thus may impact on clinical management and patient prognosis. There is a huge unmet

need for high quality research that will correlate CP aetiologies and resulting pathogenic pathways that will allow us to define quantifiable and reproducible diagnostic features of CP.

Generally, most clinicians feel that early CP cannot be confidently diagnosed based on abdominal imaging features alone. Biomarkers can objectively measure biological and pathogenic processes. There are multiple different cell types in the pancreas and CP is a complex pancreatic disorder affecting multiple systems therefore one biomarker is not representative of overall pancreatic health and cannot act as a surrogate for other biomarkers. Candidate pancreas specific biomarkers such as biochemical analytes, pain scales, imaging features, pancreatic function tests and histology may help support a diagnosis of early CP by alerting us to the fact that certain components of the pancreas are not functioning properly long before permanent radiological changes are seen. They could help determine disease stage, disease state, trajectory, and even response to treatment,

Currently there are no defined biomarkers of CP and no biomarkers that can differentiate between CP disease stage. Figure 27 below demonstrates the multiple disease stages within the progressive CP disease model. The transition points between the various stages of early, established, end-staged CP are unclear. Biomarkers would allow objective reproducible measurement of factors that define the underlying pathological process and repeated measurements over time will help assess disease activity, disease progression and response to treatment.

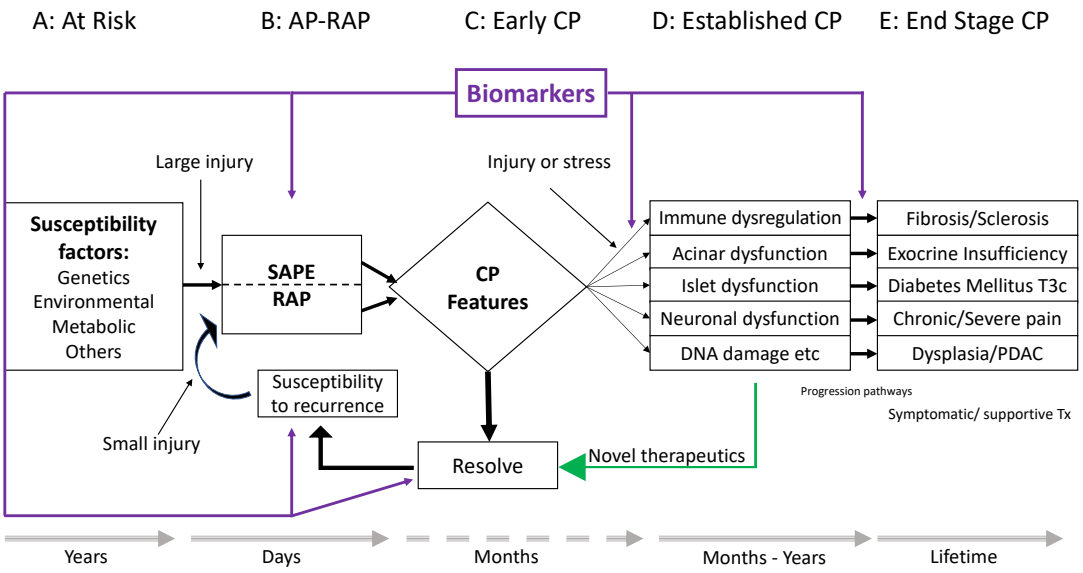


Figure 27. Time points in the natural history of CP progression where biomarkers would prove useful predictive tools

8.3 Consensus

Traditionally the management strategies for CP have heavily focussed on dealing with the sequalae of advanced and end staged disease. More recently, this focus has shifted towards ways in which the pancreatic community can avoid the disease ever progressing to an advanced stage in the first place and how to improve the dismal clinical outcomes for CP patients. To achieve this, we must find a reliable and reproducible method of diagnosing CP earlier during the potentially reversible disease stages and initiating earlier disease modifying novel interventions. This requires clinicians to understand the disease processes of CP development, progression, disease modification and treatment.

The complexity of CP and the differences in disease manifestations between countries and populations requires the perspectives of international experience and expertise. There was a strong international desire to work

together to address ‘The CP problem” and the concept of the international consensus guidelines for chronic pancreatitis was founded.

8.3.1 Future work

Whilst many of the goals of the ICGCP where achieved, consensus can be difficult to achieve in one novel process, especially in areas that have been contested and debated for long periods of time with some experts holding firmly entrenched views. The work towards consensus even in the areas where agreement could not be reached during this process has still been fruitful in that at least these areas of ongoing quagmire have been highlighted as topics that need future focus, research, and further detailed debate.

In the more challenging area of early CP, some aims were adjusted to determine whether consensus could be achieved for the definition and diagnostic criteria for early CP to identify future areas of basic, translational, and clinical research. There is a clear need to validate the new concepts and approaches towards the definition and diagnosis of early CP with large, well designed prospective clinical studies with long term patient follow up (163).

8.4 Stratifying PDAC risk in high-risk cohorts

The survival benefit for detecting PDAC early is clear and identifying pancreatic cancer as early as possible is essential. Patients diagnosed incidentally have longer median survival times than those who are diagnosed whilst experiencing symptoms and those diagnosed with stage 1 disease survive longer that those with more advanced disease (346). Survival is significantly better in patients with operable disease opposed to unresectable disease but unfortunately around 85% of patients have tumours not surgically resectable at the time of diagnosis (180). Successful early detection enables early introduction of treatments that may improve survival, symptoms, and quality of life without simply just prolonging the interval between diagnosis and death. Thankfully PDAC is relatively rare, and the general population have only a 1.3% lifetime risk of developing the disease (170). Low disease

incidence paired with an unacceptably high false-positive rate with current imaging modalities means that screening the general adult population for PDAC is unfeasible.

There are however International guidelines and a white paper supporting screening for PDAC in high-risk populations (those with a lifetime risk of the disease >5%) with the aim of identifying and treating early (T1N0M0) pancreatic cancers or high grade dysplasia in premalignant lesions such an PanINs and MCNs (223, 224). Screening individuals in high-risk groups theoretically increases the rate of detection of PDAC whilst also decreasing the rate of false positive results. Although even screening those with very high risk of PDAC such as HP PRSS1 mutations (40% lifetime risk at 70 years) has failed to demonstrate any survival benefit (223) to date.

EUROPAC screens IARs for PDAC on a research basis and the results of both the HP and FPC screening arms were presented in this thesis. Prospective data collection on individuals (and relatives) recruited to the EUROPAC database demonstrated a higher rate of PDAC diagnosis compared to the expected number in the general population thus confirming that the EUROPAC cohort are a truly high-risk population. However, this was not translated to PDAC screening yield. So, if we are screening individuals from truly high-risk populations, why are we not detecting more cases of pancreatic cancer? Are we screening the right people?

Data presented in this thesis has shown that within the already defined ‘high-risk groups’ there are further levels of risk that need to be considered when deciding which patients should be offered intensive PDAC screening. Limiting screening to only those at the highest level of risk would further enrich the screening population and theoretically increase the yield of PDAC detection.

8.4.1 Clinical implications

8.4.1.1 Strict adherence to screening protocols

Within the HP UK screening arm several clinically relevant lesions were identified during screening but unfortunately due to screening protocol deviations two pancreatic cancers were missed / had delayed diagnoses and these were the only two PDACs to be identified within this group.

The implication of the two missed/delayed diagnoses of PDAC in the HP screening cohort highlight the absolute need for strict screening protocol adherence. Entering an individual into a research screening program where there is any possibility that the individual or the screening center cannot comply fully to the agreed screening protocol may result in more harm than if that person was not undergoing screening in the first place. If complete compliance cannot be guaranteed for any reason, then screening should not be initiated.

8.4.1.2 IPMNs in FPC kindreds

Data from the EUROPAC FPC kindreds suggests that IPMN risk is not linked to familial risk of PDAC in stratified FPC kindreds. Therefore branch-duct IPMNs identified as part of high risk PDAC screening can be managed in the same fashion as BD-IPMNs identified in the general population and that existing IPMN related guidelines can be followed with no special considerations.

Factors affecting the generic risk associated with mucinous cyst progression to PDAC are still poorly understood.

8.4.1.3 Identification and consideration of other novel high-risk groups

Data presented here from EUROPAC has shown that diabetes predisposes to PDAC in HP. Yet the relationship between PDAC and diabetes is complex. In addition to those high-risk groups with inherited/genetic risk, other novel high-risk groups for the development of sporadic PDAC have been identified.

In the general population, a new diagnosis (within 1 year) of DM in is associated with 5.4 fold increased risk of developing PDAC (191) and of those

diagnosed with PDAC, up to 80% have impaired glucose tolerance (202). This is generally accepted to be due to pancreatic cancer associated hyperglycaemia and diabetes (195). Therefore, new onset DM can be an early warning sign of PDAC, thus forming a novel group of high-risk individuals for the development of sporadic PDAC.

Other novel high-risk groups include those with modifiable environmental risk factors such as obesity or smoking, those with chronic pancreatitis, and individuals with early vague symptoms. Whilst independently these factors may not represent a sufficiently large enough PDAC risk to justify screening at first assessment, careful consideration and combination within risk modelling and cancer decision support tools may identify individuals whose true risk surpasses the 5% level and thus are justified to enter PDAC screening (181, 182).

8.4.1.4 Stratifying risk and modifications to the EUROPAC screening protocols

Based on data presented here, we hypothesise that the failure of pancreatic cancer screening programmes to date can be explained by the inclusion of too many low-risk individuals in the screening study. The majority of EUROPAC's FPC families only have 2 confirmed pancreatic cancer cases and have no known causative genetic mutation, therefore DNA sequencing cannot be used to distinguish the families where PDAC clusters have occurred by chance from kindreds with a genuine autosomal dominant predisposition. Individuals from families which represent a familial cluster of sporadic pancreatic cancer cases have no elevated risk of PDAC and would not actually be eligible for screening.

The EUROPAC committee have subsequently reviewed and modified the EUROPAC screening protocols for both FPC and HP kindreds. A summary of these new protocols are given in Figures 22 and Figure 26. Family history is still a strong consideration, however more weight is given to those kindred with greater than 2 cases (to avoid recruiting potential clusters of sporadic

cases). Families with ≥3 pancreatic cancer cases may also be artefactual, but this would be progressively less common with higher numbers of affected individuals.

There is now a greater consideration of individualised risk for each participant. If eligible and the Individual volunteers for screening uptake, HP patients are then classified as requiring either relaxed or intense screening based on imaging results, biochemical markers, personal risk factors, environmental risk factors such as smoking and alcohol consumption, and the presence of diabetes. Relaxed screening is bi-annual cross-sectional imaging with annual clinical review. Intense screening is annual imaging.

The biggest change is seen with the FPC cohort where the novel Family Index and risk stratification score; Family Risk (FR) are now considered. A threshold FR of 30 has been set. Below this level a member of an FPC kindred is deemed at low risk. This is supported by the fact that no individual in our cohort has ever developed PDAC with a FR score <30. Participants classed as low risk will no longer be offered secondary screening but will continue to be included on the registry and any changes in their situation will prompt a review of their eligibility. Those with a FR > 30 will continue annual endoscopic imaging in addition to a tri-annual MRI pancreas. Those with a FR >60 will alternate EUS and MRI every six months. By refining the individuals offered screening and limiting entry into the screening program to only those at the highest risk, EUROPAC aims to further enrich the screening population and improve yield of PDAC detection with the overall aim of validating modalities for the early detection of PDAC.

8.4.2 Limitations

8.4.2.1 Patient led screening

One of the biggest potential limitations to the success of PDAC screening in high-risk individuals identified during this work relates to the process of screening uptake. As EUROPAC screens on research only basis, once an

individual is registered and eligibility confirmed the EUROPAC research team inform the patient of their eligibility to enter the screening program, but uptake from hereon in is completely led by the individual. This leaves the potential for those individuals who would be in the highest risk category to not pursue screening any further (due to any number of personal factors), whereas a highly motivated, anxious individual who is in the lowest risk category volunteering for entry into intensive screening. The prevalence of cancer in the screening population could be increased by selection based on genetic predisposition alone. However, recruited individuals in qualifying families would have first-hand experience of pancreatic cancer and fears about their personal risk can be exacerbated by the process of recruitment and registration. Counselling can be difficult as differentiating between individual and familial risk is complex (307). This issue is well demonstrated when one considers that only 10% of individuals who have their eligibility for screening confirmed come forward to volunteer for screening participation.

8.4.2.2 Lack of consensus on optimal screening methodology

There is no clear consensus on the most effective modality for the early detection of pancreatic cancer. This is attributed to ongoing technological, imaging and diagnostic developments, and secondly, the lack of outcomes seen within screening cohorts. Validation of screening methods requires observed prospective cancers within the screening group. Prospective cancers are occurring within the EUROPAC cohort of families (5 PDAC cases per year in FPC cohort and 1 PDAC case every 2 years in HP), but unfortunately these individuals were not undergoing screening.

Optimal timing of initiation and cessations of screening in high-risk individuals is largely unknown but EUROPAC advocates initiating screening at 40 years of age or 10 years younger than the youngest case of PDAC within kindreds. There is also no clear consensus on the required frequency of screening cycles and which imaging modalities are most appropriate to use. The exact

screening protocols adopted globally vary between the established screening studies, but in general include cross sectional imaging supported by blood tests including tumour markers at registration followed by annual non-ionising imaging (MRI vs EUS) with or without further tumour markers.

It is important to highlight the potential limitations of endoscopic imaging modalities when screening for PDAC on the background of CP as there is precedent within our cohort for cancer to be missed/misinterpreted as pancreatic fibrosis.

8.4.3 Future work

Data collected through cohort studies such as EUROPAC have been invaluable in helping develop a better understanding of populations at risk of PDAC. There is still much to be done to develop more accurate ways to detect PDAC at the earliest opportunity. Progress has been made with the identification of novel high-risk cohorts to further investigate such as new onset diabetes and chronic pancreatitis. There is huge scope here for the identification and validation of biological and epidemiological marker discovery and validation.

To this end, there are several ongoing trials in the USA and UK. EUROPAC continues to grow from strength to strength. Building on risk stratification work and refinement of screening presented in part in this thesis, EUROPAC PLUS aims to offer all predisposed individuals personalised pancreatic cancer screening stratified on both their family risk *and* their germline DNA risk. By incorporating individual genotyping to investigate germline DNA risk, EUROPAC PLUS will improve the efficiency of screening and the yield of early and treatable pancreatic cancer.

EUROPAC PRIME is a further initiative employing regional cancer surveillance coordinators (or ‘navigators’) to bridge the gap between individuals at higher risk of PDAC, cancer alliances, and EUROPAC thus streamlining the referral of eligible patients into surveillance services.

The UK Early Detection Initiative (UK-EDI) will recruit 2500 individuals ages 50 years and over with new-onset DM within the preceding 6 months to provide resources and data that will inform future strategies for screening individuals with new-onset diabetes for pancreatic cancer (347, 348).

The Accelerated Diagnosis of neuroendocrine and Pancreatic TumourS (ADEPTS) study is another UK based multicentre diagnostic accuracy study aiming to improve diagnostic pathways in pancreatic cancer by developing a diagnostic tool that combines refined cancer decision support tools with a minimally invasive blood test for circulating biomarkers to be used for screening in selected high-risk patient cohorts. This study will also support large, multicentre, prospective sample collection of liquid and tissue biopsies from healthy and symptomatic individuals, as well as from those known to have a genetic association or high-risk cystic lesions of the pancreas.

In the USA the Chronic Pancreatitis, Diabetes, and Pancreatic Cancer (CPDPC) Consortium was founded to collect large amounts of prospective adult and paediatric patient clinical and radiological data alongside biospecimens for diagnostic proposes and for monitoring disease status (349).

Finally, the DETECT (Evaluation of a Mixed Meal Test for Diagnosis and Characterization of Pancreatogenic Diabetes Secondary to Pancreatic Cancer and Chronic Pancreatitis) aims to investigate if it is feasible to distinguish between pancreatic ductal adenocarcinoma-associated diabetes and type 2 diabetes using a blunted pancreatic polypeptide response to a mixed meal.

The results of all these studies will be eagerly awaited and may revolutionise the approach to early detection of PDAC, especially in those with new-onset DM.

8.5 Conclusion

The complex pancreatic diseases addressed in this thesis all share a common ‘early disease phase’, presenting an opportunity for screening and initiation of treatment aiming to prevent disease progression and even the development of

pancreatic cancer. The early stages of these diseases can however be difficult to detect and have complex and highly variable clinical courses.

Progressing understanding of these complex disorders requires a paradigm shift in our approach with new focus on disease mechanisms rather than symptoms. There is a need to further stratify those individuals at risk and for further development of complex disease models. Risk stratification can also be used to optimise screening for pancreatic cancer by increasing the likelihood of early detection and mitigating the unavoidable risks associated with the screening process.

Appendix 1: Supplementary tables

1.1 Contributors to the International (EPC-APA-JPS-IAP) Consensus Guidelines for Chronic Pancreatitis process.

Table A1 Contributors for The International (EPC-APA-JPS-IAP) Consensus Guidelines for Chronic Pancreatitis.

Name	Role	Country	Society
David C Whitcomb	Chair: Whole Guidelines; Chair: Definition CP and Early CP	USA	APA
John P Neoptolemos	Co-Chair: Whole Guidelines; President EPC 2016	Germany	EPC
Tooru Shimosegawa	Co-Chair: Interventional Endoscopy; President IAP 2016	Japan	JPS
Shuiji Isaji	Surgery and timing in CP; Interventional Endoscopy President IAP 2016	Japan	JPS
Andrea Sheel	Clinical research Fellow Guideline Coordinator	UK	EPC
Carlos Fernandez-Del Castillo	Surgery and timing in CP; Interventional Endoscopy; President APA 2016;	USA	APA
Ashok Saluja	Secretary APA	USA	APA
Matthias Löhr	UEGW Guidelines	Sweden	EPC
Fiona Campbell	Pathology of CP	United Kingdom	EPC
Jens Brøndum Frøkjær	Chair: Cross sectional imaging and scoring in CP	Denmark	EPC
C Mel Wilcox	Chair: EUS in CP Surveillance for pancreatic cancer in CP Interventional Endoscopy	USA	APA
Julia Mayerle	Surveillance for pancreatic cancer in CP	Germany	EPC
Peter Hegyi	Chair: Risk Factors Secretary EPC	Hungary	EPC

Markus M Lerch	Chair: Medical Management President EPC 2018	Germany	EPC
Bill Greenhalf	Chair: Surveillance for pancreatic cancer in CP	United Kingdom	EPC
Asbjørn M Drewes	Chair: Pharmacological management of pain; Interventional Endoscopy	Denmark	EPC
Marja Boermeester	Chair: Surgery and timing in CP; Interventional Endoscopy	Netherlands	EPC
Melena Bellin	Total pancreatectomy with auto-transplantation	USA	APA
Minote Apte	Editor in chief of Peer reviewed journal Pancreatology	Australia	IAP
Marc Besselink	Total pancreatectomy with auto-transplantation Surgery and timing in CP Surveillance for pancreatic cancer in CP	Netherlands	EPC
Ingfrid S Haldorsen	Cross sectional imaging and scoring in CP	Norway	EPC
Stefan A W Bouwense	Pharmacological management of pain	Netherlands	EPC
Markus Büchler	Surgery and timing in CP	Germany	EPC
Claudia M Campbell	Pharmacological management of pain	USA	APA
Marco Del Chiaro	Total pancreatectomy with auto-transplantation Risk Factors	USA	APA
Enrique de-Madaria	Risk factors	Spain	EPC
Güralp O Ceyhan	Pharmacological management of pain	Germany	EPC
Suresh Chari	Early CP Surveillance for pancreatic cancer in CP	USA	APA
Darwin Conwell	Early CP	USA	APA
Myriam Delhaye	Pharmacological management of pain	Belgium	EPC
Ihsan Ekin Demir	Pharmacological management of pain Total pancreatectomy with auto-transplantation	Germany	EPC
J. Enrique Domínguez-Muñoz	Symptoms of CP	Spain	EPC
Mert Erkan	Surveillance in CP Surgery and timing in CP	Turkey	EPC
Irene Esposito	Chair Pathology of CP	Germany	EPC
Chris Forsmark	Early CP	USA	APA

	Surveillance for pancreatic cancer in CP		
Helmut Friess	Total pancreatectomy with auto-transplantation	Germany	EPC
Luca Frulloni	Early CP	Italy	EPC
Pramod K Garg	Early CP Pharmacology of pain Interventional endoscopy Surveillance for pancreatic cancer in CP	India	IAP
Thomas Gress	Co-Chair: Interventional endoscopy EUS in CP Surveillance in CP	Germany	EPC
Christopher Halloran	Pharmacological management of pain Surveillance for pancreatic cancer in CP	United Kingdom	EPC
Yoshiki Hirooka	Early CP	Japan	JPS
Thilo Hackert	Surgery and timing in CP Total pancreatectomy with auto-transplantation	Germany	EPC
Kazuo Inui	Risk factors in CP Medical management	Japan	JPS
Takao Itoi	Medical management Interventional endoscopy	Japan	JPS
Jakob Izbicki	Surgery and timing in CP Total pancreatectomy with auto-transplantation	Germany	EPC
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Takuya Ishikawa	Early CP	Japan	JPS
Jörg Kleeff	Surveillance in CP Surgery and timing in CP; Total pancreatectomy with auto-transplantation	Germany	EPC
Johanna Laukkarinen	Total pancreatectomy with auto-transplantation Surgery and timing in CP	Finland	EPC
Phillipe Lévy	Early CP; Surveillance in CP; Interventional Endoscopy	France	EPC

Zipeng Mark Lu	Symptoms of CP Total pancreatectomy with auto-transplantation	China	IAP
Ewa Malecka-panas	Pharmacology of pain Medical management	Poland	EPC
Atsushi Masamune	Risk factors in CP Early CP	Japan	JPS
Yi Miao	Surgery and timing in CP	China	IAP
Søren S Olesen	Cross sectional imaging and scoring in CP	Denmark	EPC
Tonya Palermo	Pharmacological management of pain	USA	APA
Steve Pandol	EUS in CP Surveillance for pancreatic cancer in CP	USA	APA
Pankaj Jay Pasricha	Pharmacological management of pain	USA	APA
Raffaele Pezzilli	Medical management	Italy	EPC
Vinciane Rebours	Medical management	France	EPC
Miklos Sahin-Toth	Risk factors President APA 2017	USA	APA
Eva Szigethy	Pharmacological management of pain	USA	APA
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Giovanni Morana	Cross sectional imaging and scoring in CP	Italy	EPC
Ihsan Ekin Demir	Pharmacological management of Pain	Germany	EPC
Ryan Baron	Surgery and timing in CP	United Kingdom	EPC
Yoshifumi Takeyama	Surgery and timing in CP	Japan	JPS
Ralph Hruban	Pathology of CP	USA	APA
Claudio Luchini	Pathology of CP	Italy	EPC
David Klimstra	Pathology of CP	USA	
Benoit Terris	Pathology of CP	France	EPC
Giuseppe Zamboni	Pathology of CP	Italy	EPC
Aldo Scarpa	Pathology of CP	Italy	EPC
Masayuki Kitano	Chair: Interventional Endoscopy	Japan	JPS
Hiroyuki Isayama	Interventional Endoscopy	Japan	JPS
Ichiro Yasuda	Interventional Endoscopy	Japan	JPS
Atsushi Kanno	Interventional Endoscopy	Japan	JPS
Kei Takase	Interventional Endoscopy	Japan	JPS
Michael Levy	Interventional Endoscopy	USA	JPS
Toshio Morohoshi	Pathology of CP	Japan	JPS
Koichi Suda	Pathology of CP	Japan	JPS
Zoltán Rakonczay	Risk factors	Hungary	EPC
Xavier Molero	Risk factors	Spain	EPC
Kyoichi Takaori	Risk factors; Surveillance for pancreatic cancer in CP	Japan	JPS
Cristian Gheorghe	Risk factors	Romania	EPC
Andrea Párniczky	Risk factors	Hungary	EPC
Jonas Rosendahl	Risk factors	Germany	EPC

Ákos Szűcs	Risk factors	Hungary	EPC
Volkan Adsay	Pathology	Turkey	EPC
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Vikas Dudeja	Total pancreatectomy with auto-transplantation	USA	APA
Martin L Freeman	Total pancreatectomy with auto-transplantation	USA	APA
Jamie D Nathan	Total pancreatectomy with auto-transplantation	USA	APA
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1.2 Clinical Contributors to EUROPAC disease registry and secondary screening study.

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