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Identification of Cystic Lesions by Secondary Screening of Familial Pancreatic Cancer (FPC) Kindreds Is Not Associated with the Stratified Risk of Cancer

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OBJECTIVES: **Intraductal papillary mucinous neoplasms (IPMNs) are associated with risk of pancreatic ductal adenocarcinoma (PDAC). It is unclear if an IPMN in individuals at high risk of PDAC should be considered as a positive screening result or as an incidental finding. Stratified familial pancreatic cancer (FPC) populations were used to determine if IPMN risk is linked to familial risk of PDAC.**

METHODS: **This is a cohort study of 321 individuals from 258 kindreds suspected of being FPC and undergoing secondary screening for PDAC through the European Registry of Hereditary Pancreatitis and Familial Pancreatic Cancer (EUROPAC). Computerised tomography, endoscopic ultrasound of the pancreas and magnetic resonance imaging were used. The risk of being a carrier of a dominant mutation predisposing to pancreatic cancer was stratified into three even categories (low, medium and high) based on: Mendelian probability, the number of PDAC cases and the number of people at risk in a kindred.**

RESULTS: **There was a median (interquartile range (IQR)) follow-up of 2 (0–5) years and a median (IQR) number of investigations per participant of 4 (2–6). One PDAC, two low-grade neuroendocrine tumours and 41 cystic lesions were identified, including 23 IPMN (22 branch-duct (BD)). The PDAC case occurred in the top 10% of risk, and the BD-IPMN cases were evenly distributed amongst risk categories: low (6/107), medium (10/107) and high (6/107) ($P = 0.63$).**

CONCLUSIONS: **The risk of finding BD-IPMN was independent of genetic predisposition and so they should be managed according to guidelines for incidental finding of IPMN.**

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INTRODUCTION

Pancreatic ductal adenocarcinoma (PDAC) is usually detected too late for curative treatment, and in 80% of patients surgical resection is not possible at the time of diagnosis [1]. Of those who undergo surgery and adjuvant chemotherapy, only 20–30% can expect to survive 5 years [2–4]. The best survival can be achieved if tumours are small at the time of resection, but such tumours are rarely found [5]. Ideally, pre-cancerous lesions could be removed before they progress. PDAC is generally accepted to arise from pancreatic intraepithelial neoplasms (PanINs), and these microscopic lesions are graded from 1 to 3, with 3 being equivalent to carcinoma in situ. However, even PanIN3 are difficult or impossible to identify by imaging. Intraductal papillary mucinous neoplasms (IPMNs) can be detected by pancreatic imaging. There is some controversy over whether IPMNs develop into PDAC, with invasive IPMN being a separate form of malignancy with a

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better prognosis [6]. PDAC do develop in patients with IPMN, sometimes after the IPMNs have been successfully resected [7], suggesting association of IPMN and cancer that goes beyond simple progression. Branch-duct (BD) IPMNs are found in at least 5% of the general population and lead to invasive cancer in a very small proportion of cases [8]; this risk is very much higher in main-duct (MD) IPMNs [9]. Mixed-type IPMNs carry a similar risk of invasive carcinoma as MD-IPMN (around 45%) [10].

Guidelines on management of IPMNs were produced in Sendai in 2006, with refinement in the Fukuoka guidelines (2012 and 2016), in essence suggesting surveillance of BD-IPMN and possible resection of MD-IPMN. The American Gastroenterological Association introduced guidelines to limit the length of surveillance of BD-IPMN; this remains controversial but enforces the idea of BD-IPMN as lesions that can usually be safely left in situ. A European guideline document has also been published, again recommending 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 [11]. Screening for pancreatic cancer requires a highly enriched population with a high prevalence of PDAC; at present, the only accepted screening populations are those with autosomal dominant predisposition for pancreatic cancer [12]. 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.

The International Cancer of the Pancreas Screening (CAPS) consortium has defined potentially relevant findings in screening as early PDAC (T1 N0 M0 R0), grade 3 PanIN, and high grade BD or MD IPMN [13]. We have reviewed 21 screening reports [14–34]; in total, 30 pancreatic cancers and 2 pancreatic neuroendocrine tumours were reported. However, the most common positive findings were cystic lesions, including 6 MD-IPMNs and 60 BD-IPMNs. Of the 1780 individuals who were screened, 131 underwent surgical resection due to what was considered to be a positive finding.

The European Registry of Hereditary Pancreatitis and Familial Pancreatic Cancer (EUROPAC) was established in 1997. EUROPAC recruits families with either hereditary pancreatitis or familial pancreatic cancer (FPC) and offers cancer screening on a research basis. This work only deals with screening in FPC. 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 of PDAC vary between 6- and 120-fold depending on the nature of family selection [35, 36], 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 paper describes the results from EUROPAC pilot screening study. A PDAC case and an 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 as a result 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.

MATERIALS AND METHODS

Patients and ethics

EUROPAC is administered from Liverpool in the United Kingdom, Greifswald in Germany and Clichy in France, mainly recruiting from Western Europe. It 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 some of the EUROPAC 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*). All screened individuals 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.

Screening protocol

Baseline measurements of serum glucose and Ca19-9 were performed alongside imaging (both pancreas protocol computed tomography and endoscopic ultrasound of the pancreas). The screening process is summarised in Fig. 1. Consenting individuals had collection of duodenal juice, with secretin stimulation. If the juice contained no cancer-associated genetic abnormalities, participants entered a 3-yearly screening cycle, with staggered endoscopic ultrasound (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. Abnormalities were discussed at the supra-regional pancreatic multi-disciplinary team meeting.

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

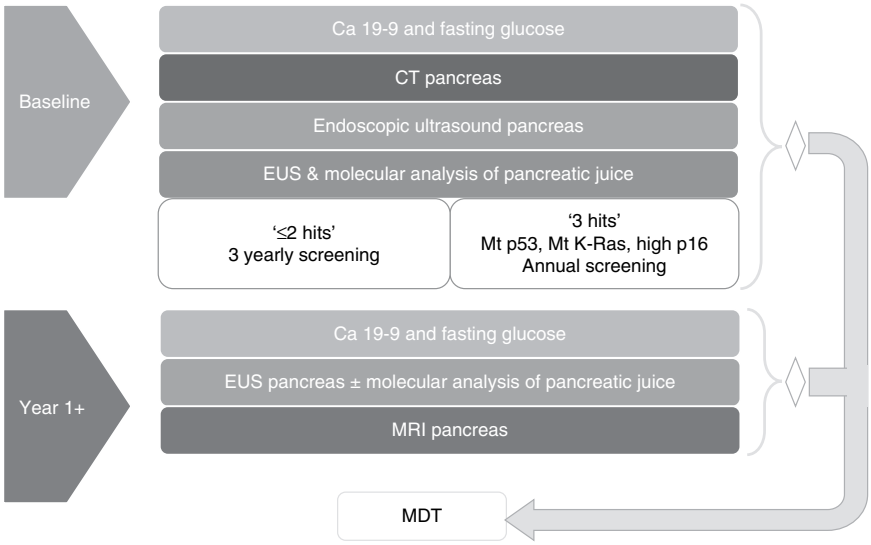

Fig. 1 EUROPAC screening protocol. There is a 3-yearly screening cycle following a baseline assessment consisting of computed tomography (CT), endoscopic ultrasound (EUS) and blood tests. There is EUS imaging at the end of each cycle, followed by collection of pancreatic juice (previously this was done by cannulation of the pancreatic duct, currently by collection from the duodenum following secretin stimulation) 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/magnetic resonance imaging (MRI). 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 whether the participant undergoes annual surveillance or regular clinical review and/or follow-up

molecular analysis has been previously described [37]: 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 manufacturer's protocols [38], and in order to avoid false positive results from PCR error, the template DNA was diluted to 10 genomes per reaction prior to making each library. Minimum allele frequency for a genuine mutation would therefore be approximately 10, and a 1% mutation rate would be 1 out of 10 libraries from the same patient with a mutant allele frequency of 10% and the rest wild type.

Risk calculation

The purpose of this study was to evaluate the relationship between screening results and familial predisposition, and 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. The concept of family index (*FI*) was used, 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.

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

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.

Statistical analysis

Continuous variables are presented as median with interquartile 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's chi square or Fisher's exact testing as appropriate.

RESULTS

The demographics of all individuals consented and recruited to the FPC EUROPAC registry are described in Table 1. The families are split according to number of pancreatic cancer cases and generations affected.

In Table 2 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 1. 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.

The prospective cancer cases are described in Table 3. 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 1 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. 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.

Table 1 Demographics of EUROPAC-registered individuals and the cancer cases in their families in October 2016 for comparison with the screened cohort

Family type	Total kindreds	Total number of PDAC in kindreds	Registered individuals				Number of individuals screened
			Total registered Individuals	Current age range/ median	Gender	Smoking	
FPC ≥3 cases multi-generations	145	504	308	24–99/ 58	F = 195 M = 113	Yes = 41 No = 145 Ex = 94 Unknown = 28	84 Individuals 60 Families
FPC 2 cases 2 generations	281	562	438	24–91/56	F = 275 M = 163	Yes = 43 No = 211 Ex = 135 Unknown = 49	130 Individuals 106 Families
FPC ≥3 cases 1 generation	25	80	44	32–85/57	F = 27 M = 17	Yes = 7 No = 13 Ex = 14 Unknown = 10	6 Individuals 6 Families
FPC 2 cases 1 generation	110	220	190	25–91/57	F = 130 M = 60	Yes = 26 No = 97 Ex = 56 Unknown = 11	48 Individuals 40 Families
BRCA2 mutation	54	39	81	28–84/57	F = 58 M = 23	Yes = 4 No = 37 Ex = 32 Unknown = 8	22 Individuals 20 Families
FAMMM	14	24	27	34–67/49	F = 19 M = 8	Yes = 7 No = 15 Ex = 4 Unknown = 1	9 Individuals[a] 8 Families
PJS	6	2	7	19–69/44	F = 2 M = 5	Yes = 1 No = 3 Ex = 2 Unknown = 1	4 Individuals 4 Families
HNPCC	15	21	20	41–76/60	F = 12 M = 8	Yes = 0 No = 9 Ex = 7 Unknown = 4	8 Individuals[b] 5 Families
Other[c]	66	83	84	30–92/59	F = 55 M = 29	Yes = 8 No = 43 Ex = 22 Unknown = 11	10 Individuals 9 Families
Total	716	1535	1199	19–99/57	F = 773 M = 426	Yes = 137 No = 573 Ex = 366 Unknown = 123	321 Individuals 258 Families

EUROPAC European Registry of Hereditary Pancreatitis and Familial Pancreatic Cancer, *F* female, *FAMMM* familial atypical multiple mole melanoma, *FPC* familial pancreatic cancer, *HNPCC* hereditary non-polyposis colorectal cancer (Lynch Syndrome), *M* male, *PDAC* pancreatic ductal adenocarcinoma, *PJS* Peutz Jegher's syndrome
[a]Four individuals were recruited for screening from a family with a CDKN2a mutation but were later found to not be carriers
[b]Four individuals with MLH1 mutations and 4 defined on family history alone
[c]Families with cancer syndromes (none with known causative mutations)

Table 2 Individuals in EUROPAC kindreds followed from 2000 to 2016 showing prospective cancers to demonstrate high risk

	All individuals (registered or unregistered relative)				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 2 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

EUROPAC European Registry of Hereditary Pancreatitis and Familial Pancreatic Cancer, *IQR* interquartile range

Table 3 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=1Unknown=0	1.0 (0.8–1.4)	48.5 (41.0–69.7)
Single generation 2 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)

FI family index, *IQR* interquartile range

EUROPAC had recruited 321 individuals for screening from cancer families up to October 2016 (321/3031 10.6% of all eligible individuals). In all, 123 participants had completed more than two 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 indi-

Table 4 Screening events stratified by risk group

	Low risk	Medium risk	High risk
Median age (IQR): whole group	58 (47–65)	52 (46–61)	53 (48–63)
Smoking: whole group			
Yes	5 (4.7%)	11 (10.3%)	5 (4.7%)
No	56 (52.3%)	60 (56.1%)	65 (60.7%)
Ex	46 (43.0%)	36 (33.6%)	37 (34.6%)
PDAC	0	0	1
pNET	1	1	0
MD-IPMN	0	1	0
BD-IPMN	6	10	6
Size of BD-IPMN			
3–5 mm	3	7	4
6–10 mm	1	3	2
>10 mm	2	0	0
Progression of BD-IPMN			
Progressed	2	1	0
Stable	3	7	4
Regressed	1	2	2
Other cystic lesions	5	6	7
Median age (IQR): individuals with cystic lesions	60 (54–65)	52 (48–70)	59 (47–67)
Smoking: cystic lesion group			
Yes	1 (9.1%)	2 (11.8%)	0
No	6 (54.5%)	9 (52.9%)	7 (53.8%)
Ex	4 (36.4%)	6 (35.3%)	6 (46.2%)
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

BD branch duct, *IQR* interquartile range, *IPMN* intraductal papillary mucinous neoplasm, *MD* main duct, *PDAC* pancreatic ductal adenocarcinoma, *pNET* pancreatic neuroendocrine tumour

viduals who had been recruited and undergone baseline screening but with less than 1 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 4 and Fig. 2. 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 IPMNs. 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 neoplasias. Two pancreatic neuroendocrine tumours (pNETs) were discovered, both were resected and were found to be well differentiated. One PDAC was identified. In addition, a gastrointestinal stromal tumour 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.

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

Of the 35 screened individuals with a known causative mutation, only 2 had a significant finding on screening; 1 out of 22 with a *BRCA2* mutation had a 10 mm BD-IPMN that regressed during clinical follow-up, and 1 out of 5 individuals with a *CDKN2a* mutation had an 11 mm BD-IPMN which has remained stable after 36 months of follow-up.

Only 48 participants consented for endoscopic retrograde cholangiopancreatography with molecular analysis of pancreatic juice and only 4 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 two separate pancreatic juice collections. The first gave wild-type *KRAS* and normal levels of *CDKN2a* promoter methylation (0.01%), and *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, and in a previous analysis they had wild-type *KRAS* and *Tp53* with normal *CDKN2a* promoter methylation (0.018%).

In Fig. 2 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, and 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 years, giving an FI of 1.179. There was a 50% chance of the individual being a carrier

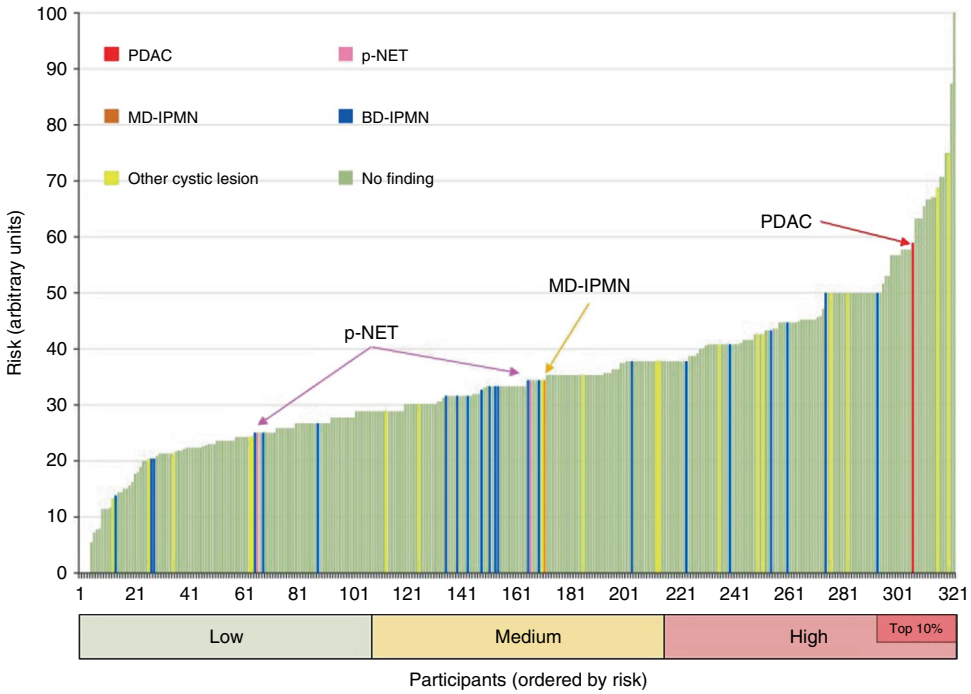

Fig. 2 Screening results stratified by risk that an individual carries a high penetrance mutation predisposing to pancreatic cancer. Risk was estimated based on number of cases of cancer, number of at-risk individuals in each family and the chance that a participant was carrying an autosomal dominant 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

and 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 Fig. 2 indicated no correlation between risk and incidence for branch-duct IPMN ($\chi^2 = 0.937$; $P = 0.632$).

DISCUSSION

The screening described here was carried out under the assumption of autosomal dominant predisposition for PDAC. 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 [39]. 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.

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 IPMNs were identified with equal probability in individuals of all familial risk categories.

The 5-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%), and hence our data are entirely consistent with the expected discovery of cystic lesions in the general population [40]. Age is a risk factor both for the development of pancreatic cancer and IPMN (as shown in the SHIP analysis), and 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 (see Table 3) 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.

There is little doubt that BD-IPMNs are associated with cancer risk. However, although an individual with a BD-IPMN may be at greater risk of cancer, our data suggest individuals with a higher inherited risk of PDAC are not at a higher risk of developing BD-IPMN. Previously, Capurso et al. [41] showed that IPMNs were more frequent in individuals with a familly history of pancreatic cancer, and this was based on 21 IPMN cases (5.4 %) with a first-degree family history of PDAC compared to 6 controls (1.6 %), but only 1 of these would have fitted the criteria for FPC and this patient still only had 1 first-degree relative with PDAC (plus 2 second-degree relatives). Our findings indicate that FPC is not associated with greater predisposition for IPMN; genetic predisposition for IPMN may well be associated with higher risk of PDAC. Similarly, patients who smoke or who have diabetes may well be more likely to develop IPMN and be more likely to develop cancer.

The link between genetic predispostion to cancer and to precursor lesions is complex, and syndromes such as familial adenomatous polyposis 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 [42]. In contrast, Lynch Syndrome (or hereditary non-polyposis colorectal cancer (HNPCC)) increases the risk of precursor lesions progressing [43], and hence 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. There is emerging support for an alternative to the traditional progression model. A catastrophic process of cancer development associated with Acinar Ductal Metaplasia and lobular atrophy, independent of PanIN [44, 45], fits with observations in familial pancreatic cancer [46]. Atypical flat lesions may be positive screening results on the basis that they are the pre-cancerous lesion typical for FPC [47], but unfortunately, these cannot be identified without first resecting the pancreas.

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.

The screening results presented here are consistent with the outcomes described by other groups, with discovery of cystic lesions far outweighing identification of PDAC [14–34]. 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. [39] developed the PancPro Mendelian model to identify high-risk individuals within FPC kindreds. 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 [35], with the added advantage of stratifying risk within groups of families with equal numbers of pancreatic cancer cases (see Table 3). Although Table 3 shows that the prospective cancer cases have a higher FI than equivalent individuals in our screened cohort, who have not so far developed cancer, this cannot be considered as validation of FI as a concept as in order to do this we would have to show greater risk of cancer in a prospective cohort of individuals with standardisation for all other risk factors (smoking, age, diabetes etc.). We are carrying out such a prospective analysis with the families shown in Table 1, but these data will not be available for some years. 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.

If we had identified an increased frequency of IPMN in higher-risk individuals, we could have reasonably concluded that FPC predisposes to the development of IPMN which in turn predisposes to PDAC, but this was not the case. If we had found that IPMNs encountered during screening progressed to PDAC, we could have reasonably concluded that FPC increases the probability of an IPMN progressing, but again this was not the case. We cannot conclude from this work that IPMNs are an intermediate stage in the development of PDAC within FPC kindreds. IPMNs identified during screening should on this basis be treated in the same way as IPMNs 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 IPMNs.

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CONFLICT OF INTEREST

Guarantor of the article: W. Greenhalf.

Specific author contributions: EUROPAC was established in 1996, the lead clinician is CH. The lead scientist for EUROPAC is WG supported by EC. SH supported the description of family trees including ascertaining diagnosis. The initial concept and study design included WG, EC and MML. ARGS, IS, JAN, CH, CG, MR, MC, AS, RC, CM, ZH, GPA, PH, MML and SPP supported patient recruitment and surveillance. JR provided expertise in endoscopic procedures and AF provided expertise in radiological procedures. RJ provided statistical support. The initial draft was written by ARGS, WG and IS. All authors contributed to the final version of the paper.

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Study Highlights

WHAT IS CURRENT KNOWLEDGE

- ✓ There are families with multiple cases of pancreatic cancer suggesting autosomal dominant predisposition (true FPC).
- ✓ Clusters of pancreatic cancer cases will occur by chance giving little prospective increased cancer risk.
- ✓ Screening of pancreatic cancer families frequently yields pancreatic cystic lesions but not many cancers.
- ✓ Some sporadic pancreatic cystic lesions lead to cancer but most remain indolent.
- ✓ It is unknown whether FPC influences either the incidence or progression of cystic lesions.

WHAT IS NEW HERE

- ✓ Stratification by family history makes no difference to yield of cystic lesions within FPC kindreds.
- ✓ BD-IPMNs may be incidental and so cannot be taken as a positive screening outcome.
- ✓ BD-IPMNs found during screening should be managed in the same way as those found incidentally.

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