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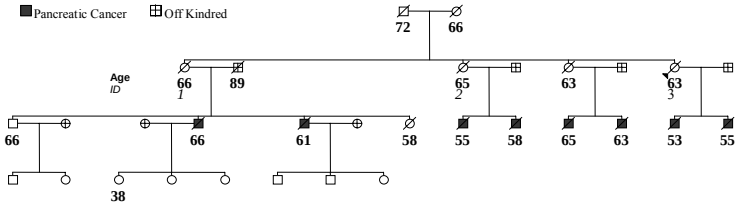
Definition and characterisation of FPC families

It is very difficult to formulate rules for the definition of an FPC family, simple rules based on the number of cases or generations involved are not adequate. Three family trees of FPC families on the EUROPAC registry and a family not considered to be FPC are shown in Figure S1. Family 1 would be considered consistent with FPC due to the large number of pancreatic cancer cases which make a chance occurrence seem unlikely. Individual 1 and her sisters died below the age of 70 so it is not unreasonable to consider them as non penetrant – particularly as individuals 2 and 3 may well have died of pancreatic cancer, but no histology or good quality medical notes were available to confirm this. Family 2 has two cancer cases, but either of individuals 1 or 2 could be non penetrant.. The other generations in this family tree are entirely consistent with full penetrance. We would not consider family 3 as consistent with autosomal dominant inheritance as it only has two affected cases, and individuals 1 or 2 have to be non penetrant at quite an advanced age, the multiple siblings of individuals 1 and 2 would have to be either non penetrent or non-carriers and one of their parents (individuals 5-8) would also have to be non-penetrant at an advanced age. Although Family 4 is similar to Family 3, the family tree is less extensive and one of the parents died at a young age, in this case the family was classified as being a potential FPC family. This subjective approach to family categorisation is clearly open to error and some families classified as FPC will have occurred by chance clustering of affected individuals, but classification just on the number of pancreatic

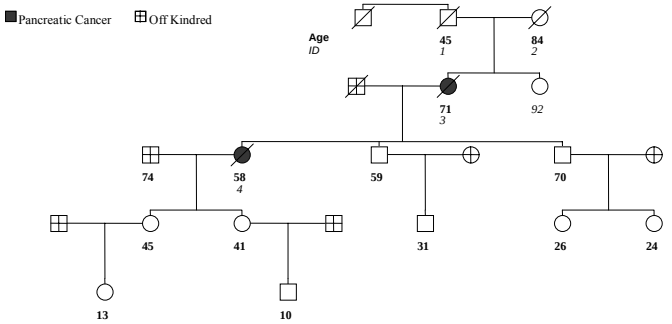
cancer patients in a family will exclude many genuine FPC families that happen to have small kindreds.

Statistics on the families chosen in this study are given in the tables below. All families were characterised over at least 3 generations As it happens all families and all subdivision of families (haplotyped and excluded, haplotypes and not excluded, haplotyped with no conclusion possible and just allelotyped) had a median of 4 characterised generations.

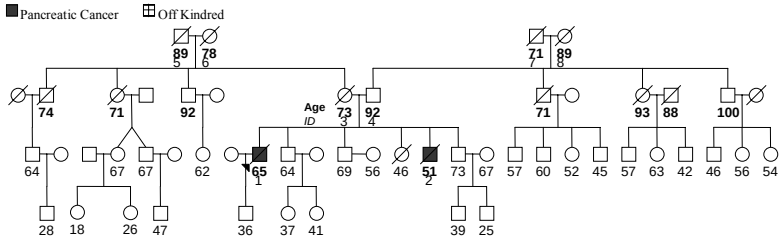
Figure S1: Classification of FPC



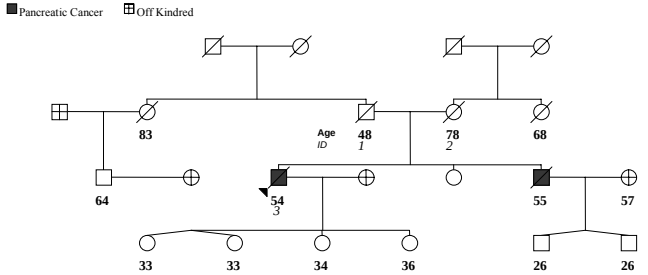
Family 1



Family 2



Family 3



Family 4

Family 1, 2 and 4 would be classified as FPC, but family 3 was not used in this study because of inadequate evidence of autosomal dominance

Table S1: Number of individuals broken down by result of analysis

	Number of Families	Number of affected: Median per family: (Range): Mean per family	Number unaffected (on-kindred) Median per family: (Range): Mean per family
Allelotyped	70	184: 2(2-10): 2.6	1410: 20(7-51): 15
Haplotyped (Excluded)	8	18: 2(2-3): 2.3	195: 26(17-40): 24
Haplotyped (Possible)	8	22: 2(2-6): 2.8	146: 20(7-32): 18
Haplotyped (No-conclusion)	25	67: 2(2-10): 2.7	854: 20(7-51): 34

The range of affected individuals per family, details on gender and the age of cancer onset are given in Table 4 of the main paper.

Table S2: Families broken down by nationality

Nationality	Allelotyped Families (affected:unaffected)	Haplotyped (excluded) Families (affected:unaffected)	Haplotyped (Possible) Families (affected:unaffected)	Haplotyped (No-conclusion) Families (affected:unaffected)
UK	49 (125:675)	4 (9:119)	6 (17:104)	11 (27:249)
Irish	1 (3:12)			
German	12 (25:386)	3 (6:56)		9 (19:330)
Hungarian	3 (9:173)			3 (9:173)
Italian	4 (19:132)	1 (3:20)	1 (3:10)	2 (12:102)
Swedish	1 (3:32)		1 (2:32)	

Table S3: Form of cancer confirmation

	Number of affected confirmed by histology (a)	Number of affected confirmed by medical notes (b)	Number of affected confirmed by cancer registry
Allelotyped	79	71	34
Haplotyped (Excluded)	11	4	3
Haplotyped (Possible)	11	9	2
Haplotyped (No-conclusion)	32	26	7

If histology was available this was taken as the gold standard (a); where histology was not available or ambiguous a decision on definition of ductal adenocarcinoma was made using medical notes (b); where medical notes were not available inclusion on a cancer registry as having pancreatic cancer was considered adequate confirmation for inclusion (c)

Allelotyping

Allelotyping was carried out using nine mapping pairs between D4S413 and D4S415 spanning a 23-*cM* region of chromosome 4q32-34. PCR products were sized using the ABI377 DNA sequencer and/or the capillary based 3100 ABI Genetic Analyser. The data were analysed and the alleles were assigned by Genotyper software (ABI). A marker of a defined size will incur an altered mobility depending on whether it was analysed using a gel (ABI377) or capillary (ABI3100) based system. Therefore we analysed all nine microsatellite markers of a known size from sequenced BAC clones on both systems and calculated and corrected the discrepancy between the two systems. Primers were obtained from the ABI prism linkage mapping set version 2 or were custom synthesised by MWG-Biotech (Table S4).

Table S4: Details of the microsatellite markers

Marker	GenBank Accession number	Heterozygosity (%)	Size range (bp)	No of alternative alleles	Forward primer sequence	Reverse primer sequence	Label
D4S413	Z16837	90.0	227-320	12	ABI Prism Linkage set version 2	ABI Prism Linkage set version 2	FAM
D4S393	Z16459	92.31	104-118	7	GAATCCCCAAGCACAATGTC	TGGCTGTTTTTGGATGCTAC	HEX
D4S1603	Z24246	67.86	190-208	8	CAAATATGTCATTTTATATGCTG	TGGTGTGTCTCTGATGCT	HEX
D4S2952	Z51120	67.86	181-203	7	AGTCTCAGTTCCTCACAGGC	TAGGTAGATTCCAAAATGACCTCC	FAM
D4S1596	Z24149	60.17	213-223	4	CTTCTTATTCCCATGCCAC	TGTTCACTAGGCCTTACATCTCA	HEX

Table S4: Details of the microsatellite markers (continued)

Marker	GenBank Accession number	Heterozygosity (%)	Size range (bp)	No of alternative alleles	Forward primer sequence	Reverse primer sequence	Label
D4S1597	Z24158	82.14	273-293	10	ABI Prism Linkage set version 2	ABI Prism Linkage set version 2	NED
D4S1617	Z24575	53.57	181-191	6	ATTGACTGAGTTTTTACAGGG	TCTGGTTTGATATTTGTGTG	HEX
D4S1539	Z23446	64.29	221-229bp	5	ABI Prism Linkage set version 2	ABI Prism Linkage set version 2	FAM
D4S415	Z16841	85.71	172-202bp	10	ABI Prism Linkage set version 2	ABI Prism Linkage set version 2	NED

Candidate gene sequencing

The entire D4S413-D4S315 region was scrutinized in order to identify candidate FPC genes. Within this region, several genes were identified that may be associated with cancer, including growth regulators such as vascular endothelial growth factor C(S1), annexin A10 which has been shown to be down regulated in hepatocellular carcinoma(S2). However, these were considered to be unlikely candidate FPC genes as a mutation would aid tumour progression rather than have a causative effect.

Four genes within the locus were chosen as being of particular interest. Cyclophilin D/Cyclophilin 40 (PPID) is a component of the mitochondrial permeability transition pore and the gene is located between markers D4S413 and D4S393. Inhibition of cyclophilin D hyperpolarizes the mitochondrial membrane potential and inhibits apoptosis, thus a mutation might similarly inhibit apoptosis and so be tumourogenic(S3). PPID is also associated with the cancer related stress protein Hsp90 and involved in targeting Hsp90 to inactive glucocorticoid receptor(S4) and dynein(S5). Mortality factor 4 (MORF4), thought to transcriptionally modulate genes important for senescence pathways or cell growth control(S6) and Tetican 3 (SPOCK 3) a metalloendopeptidase inhibitor with calcium binding activity that interferes with tumour invasion in brain(S7) also map to this region. One gene involved in DNA repair was identified within the locus, High Mobility Group Box 2 (HMGB2), which maps in close proximity to D4S1617. HMGB2 acts in an alternative DNA damage detection system to MutS homologues(S8).

DNA from 10 affected individuals from 10 different families was used for sequencing of candidate genes within the 4q32-34 locus. In addition, these genes were sequenced in pancreatic cancer cell lines Capan-2, Panc-1 and FAMPAC, the latter cell line being isolated from a patient with a familial predisposition to pancreatic cancer(S9). The primers used are shown in Table S5.

No mutations or polymorphisms were identified in the *HMGB2*, *PPID* or *MORF4* genes in the 10 individuals and three cell lines used. Full length sequences of *SPOCK3* were only obtained for five patients, including representatives from FamJ of

the D4S1617-186 group and FamU of the D4S1597-282 group. No mutations were identified although several known SNPs in both coding and non-coding regions were observed.

Table S5: PCR and sequencing primers used in gene sequencing.

Exon (size product bp)	Forward primer	Reverse primer
HMGB2		
PCR Primers		
1 (335bp)	GCCGAGACCGAGTAGAGGCT	AGACACTGACCAATCGTTAG
2 (327bp)	TCGTCTGCCTGGAACTCTTA	GCCGAGACCGAGTAGAGGCT
3 (533bp)	TTCCCAGGTTTTCTACAGTT	TCTTGTCGTCTTTGGATGTT
5 (706bp)	CTAGGTCAACTGTGTCTAAG	GAGTAATGATACCGGATGCT
Sequencing Primers		
1	CGTCAGAACCAATCAGATTA	
2	TCATGAGTTGCCTACTACCT	
3	TATAGGTAACAATTTTGCTGTATTT	
4	GGTTTTCTACAGTTATTACCTATAC	
5	GGTCAACTGTGTCTAAGAACTACTA	
5	ATGATACCGGATGCTT	
PPID		
PCR Primers		
1 (185bp)	CGTCAGACTCCGCTCACCTC	CGGAGACGGACTCTGGAGTT
2 (195bp)	AACAGGACTTGTCTGTATGA	AGTTGGTCGAATTGTCTTAG
3 (224bp)	GTGTCTTAATCCAACAAGTA	TCCATGTCTCAGTCACTAAT
4 (272bp)	GCCTGGAACAGATCGAGACT	CAGCATGATCGGGAGGGTTT
5 (485bp)	GAGCAATTCTTAATTTTAGT	CTACAATTTAGGGTTATTTT
7 (257bp)	AGTCTTAATCCAACTTATTC	TGGACATAGAGAAGATGAAT
8 (1260bp)	AGGGATCATATTCATAGACA	TTGAAATCTGGCATATAAGC
Sequencing Primers		
8	CTTAATACTTTGATATAAAT	
9	CAGGCATTCTGTAGTGTCTG	

Table S5: PCR and sequencing primers used in gene sequencing (continued).

Exon (size product bp)	Forward primer	Reverse primer
MORF4		
PCR primers		
1 (989bp)	ATCCCTGTGTTGTCCATCAT	AAAGCCAATCAGGAGCAGTA
Sequencing primer		
1	TCCGATGGTACTCAGGAAGA	
SPOCK3		
PCR primers		
1-2 (916bp)	TCCTATCCGGAGCCAACTGT	GGCAGCGGAACTGTGAACTT
3-4 (800bp)	TCCATGGGCCACTGCAGAAT	GGTTCAGAAGCCCAAATCAG
5 (457bp)	GGCAGTAGCAACTACAGAGA	GTAAGGAGCGCTGTTCAATC
6 (1087bp)	TAGGCACCCAGTGGATTAGA	GGGACACTTAATTGGGAGGAAC
7 (980bp)	GTGCAAGTCACTGGCTAAAG	ACTGAGCAGTTGCAGATC
8 (742)	CAAGAGGTATGGCCAACA	ACACCCTCTGAGGAGACATT
9 (575bp)	GCTACCTGCTATGTTGAC	TGCTTCTTGGTGGCTCAT
10 (490bp)	GCTTCAGCAGCAGTCAAT	CACCTAGGAGCTTCTTTACC
11 (356bp)	GCATAATGGGTGCTTCTCAG	TCCCTCCACCCTAGATATTC
12 (2103bp)	TCTGCCACACTTGTAGAAGA	TGAGAAGGCTTCATGCTACT
Sequencing primers		
12	GATGGCAGTCTCATGCAGAT CCTAAGTTGCTGGTCAGA	

Two-point LOD score analysis

Two point LOD score analyses were performed using parametric two-point analyses with the MLINK program from the LINKAGE (version 5.1) software package(S10) with three different models. The LOD scores were calculated on the basis of the age related penetrance and the probability of being a carrier based only on relationship with affected individuals. Models 1 and 2 assumed an autosomal dominant mode of inheritance with a very low global rate for phenocopies (0.001) and an age-dependent penetrance (as above) in order to account for carriers who have not developed cancer. The age-dependent penetrance was implemented by using varying numbers of age related liability classes, 20 classes were used for model 1 and six classes for model 2. Global penetrances were used for model 3 as only affected individuals were taken into account. Allelotypes of 196 on-kindred individuals from 70 families were used. Families with known BRCA2 mutations and two families where the only allelotypes were from participants with no first degree relative with pancreatic cancer were excluded. Two-Point-LOD score analysis of all 70 families permitted exclusion mapping for each marker. The results of LOD score analyses are shown in Figures 2 of the main paper and in Table S6a-c. LOD scores for each marker are plotted against cM distance (Marshfield map(S11)) of the locus as a function of the recombination frequency. LOD scores were plotted to a maximum distance of 60cM from D4S413 due to the fact that the end of chromosome 4 was approximately 53cM telomeric of D4S413.

Table S6: Two-point-LOD score analyses

a MODEL 3 LOD Score at θ =

Marker	0	0.01	.05	0.1	0.2	0.3	0.4
D4S413	-2.02						
D4S393	-1.20		-0.68	-0.43	-0.18	-0.06	-0.01
D4S1603	-1.01	-0.86	-0.52	-0.29	-0.06	-0.03	-0.05
D4S2952	-0.05			-0.05	-0.03	-0.01	-0.002
D4S1596	-0.003	-0.02	-0.06	-0.09	-0.12	-0.11	-0.07
D4S1597	-0.27	-0.25	-0.20	-0.15	-0.08	-0.03	-0.01
D4S1617	0.273	0.271	0.26	0.24	0.19	0.14	0.07
D4S1539	-0.45	-0.42	-0.33	-0.24	-0.12	-0.05	-0.01
D4S415	0.28	0.28	0.27				

b MODEL 2 LOD Score at θ =

Marker	0	0.01	.05	0.1	0.2	0.3	0.4
D4S413	-4.47		-1.96	-1.09	-0.29		
D4S393	-7.34		-2.73	-1.68	-0.68	-0.24	-0.05
D4S1603	-3.13	-2.60	-1.62	-0.99	-0.33	-0.03	-0.06
D4S2952	-1.78	-1.55	-0.94	-0.52	-0.13	-0.003	-0.01
D4S1596	-0.67	-0.60	-0.38	-0.19	-0.03	-0.10	-0.08
D4S1597	-2.47	-2.30	-1.77	-1.29	-0.65	-0.26	-0.06
D4S1617	-0.11	-0.09	-0.001	0.07	0.14	0.13	0.08
D4S1539	0.75	0.73	0.64	0.53	0.31	0.13	0.03
D4S415	0.59	0.65	0.81				

c MODEL 1 LOD Score at θ =

Marker	0	0.01	.05	0.1	0.2	0.3	0.4
D4S413	-4.98		-2.51	-1.62			
D4S393	-7.04		-2.80	-1.82	-0.82	-0.32	-0.08
D4S1603	-2.80	-2.38	-1.62	-1.12	-0.54	-0.22	-0.05
D4S2952	-2.43	-2.17	-1.49	-1.00	-0.45	-0.17	-0.04
D4S1596	-1.15	-1.08	-0.85	-0.62	-0.30	-0.12	-0.03
D4S1597	-2.31	-2.07	-1.47	-1.02	-0.49	-0.20	-0.05
D4S1617	0.00	0.022	0.07	0.09	0.07	0.04	0.01
D4S1539	-2.26	-1.38	-0.72	-0.43	-0.17	-0.06	-0.01
D4S415	-3.19	-2.22	-1.31	-0.83	-0.35	-0.13	-0.03

a): Model 3 using a global penetrance function as only affected individuals are taken into account. b) Model 2 assuming an age-dependent penetrance and six liability classes. c) Model 1 assuming an age-dependent penetrance with two risk categories, (low and high) and 20 liability classes.

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