Contribution of known and novel DNA repair genes to pancreatic cancer susceptibility

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Abstract

Pancreatic adenocarcinoma (PAC) is a deadly malignancy that most commonly presents at a late stage and is ultimately refractory to systemic therapies. Even more devastating is the familial clustering of PAC (and other cancers) that is observed in 10-15% of cases. These kindreds, however, represent an opportune subset of patients for studies aimed at early detection and precision oncology strategies. While a fraction of the observed familial clustering of PAC is attributable to inherited (i.e., germline) genetic mutations in known PAC susceptibility genes (e.g., *BRCA1* and *BRCA2*), the genetic causes for the overwhelming majority of familial PAC (~85%) remain undefined. The rapid lethality of PAC has hindered the collection of DNA, tumour specimens, and high-quality clinical and epidemiologic data that are critical for genetic studies of PAC. Herein, we demonstrate that a rapid ascertainment methodology, as used by the Quebec Pancreas Cancer Study (QPCS), a prospective clinic-based PAC research registry that was established in our lab in 2012, leads to high participation rates and allows for the collection of rare "high-risk" kindreds for studies of PAC heredity. Motivated by recent studies by our group and others suggesting that PAC associated with germline mutations in homology-directed DNA repair (HDR) genes have increased sensitivities to DNA damaging agents (e.g., platinums) and poly(ADP-ribose) polymerase inhibitors, we combined the resources of the QPCS and the Ontario Pancreas Cancer Study to define the prevalence of germline mutations in 4 known HDR-implicated PAC susceptibility genes (*BRCA1*, *BRCA2, PALB2* and *ATM*) among 150 consecutive PAC cases with French-Canadian ancestry – a population known to harbour founder (i.e., recurrent) mutations in these genes – and 236 cases unselected for ancestry. Using clinical data collected by these registries, we provide supporting evidence for the role of precision therapy in this PAC subtype, and make recommendations for reflex genetic testing that can be easily applied in the routine

management of PAC. To elucidate the unexplained majority of familial PAC, we used next-generation sequencing to interrogate the germline exomes of 109 PAC cases from 93 "high-risk" kindreds and used a filter-based approach to identify several candidate PAC susceptibility genes involved in DNA repair. Most notable are *FAN1, NEK1* and *RHNO1*, which each harboured pathogenic mutations in 3 kindreds (3.2%) and demonstrated segregation with PAC in 2 kindreds. The identification of several low prevalence candidate genes, rather than a single major gene in our large case series highlights the likely heterogeneity of PAC heredity. Adverse survival was observed in early stage PAC cases with germline mutations in DNA repair genes, pointing to a hypothesis that these cases may represent a distinct clinical subtype with selective drug sensitivities. Overall, this thesis aims to characterize the contribution of both known and novel germline genetic causes of PAC and supports the notion that distinct genetic subtypes of PAC may benefit from targeted therapies, highlighting the limitations of the current "one-size-fits-all" approach to PAC treatment.

Résumé

L'adénocarcinome pancréatique (ACP) est un cancer mortel qui se présente le plus souvent à un stade avancé et finit par être réfractaire aux thérapies systémiques. Encore plus dévastatrice est l'agrégation familiale de l'ACP (et d'autres cancers) qui est observée chez 10-15% des cas. Cependant, ces apparentés représentent un sous-ensemble opportun de patients pour des études visant la détection précoce et les stratégies d'oncologie de précision. Alors qu'une fraction de l'agrégation familiale observée de l'ACP est attribuable aux mutations génétiques héréditaires (c.-à-d., germinales) dans les gènes de susceptibilité à l'ACP connus (ex., *BRCA1* et *BRCA2*), les causes génétiques de la majorité de l'ACP familial (~85%) restent indéfinies. La mortalité rapide de l'ACP a entravé la collecte d'ADN, de spécimens de tumeurs et de données cliniques et épidémiologiques de haute qualité qui sont essentielles pour les études génétiques de l'ACP. Nous démontrons ici qu'une méthodologie d'inspection rapide, telle qu'utilisée par l'Étude Québécoise sur le cancer du pancréas (QPCS), un registre prospectif de recherche sur les ACPs établi dans notre laboratoire en 2012, conduit à des taux de participation élevés et permet de rassembler de rares cas d'apparentés «à haut risque» pour des études sur l'hérédité de l'ACP. Suivant des études récentes de notre groupe et d'autres groupes suggérant que l'ACP associé aux mutations germinales dans les gènes de réparation homologue directe de l'ADN (RHD) ont une sensibilité accrue aux agents endommageant l'ADN (ex. platines) et inhibiteurs de poly(ADP-ribose) polymérase, nous avons combiné les ressources du QPCS et de l'Étude Ontarienne sur le cancer du pancréas pour définir la prévalence des mutations germinales dans 4 gènes connus de susceptibilité à l'ACP impliqués dans la RHD (*BRCA1*, *BRCA2*, *PALB2* et *ATM*) parmi 150 cas d'ACPs d'ascendance canadienne-française – une population connue comme ayant des mutations fondatrices (c.-à-d. récurrentes) dans ces gènes – et 236 cas non sélectionnés pour l'ascendance. En utilisant les

données cliniques recueillies par ces registres, nous apportons des preuves du rôle de la thérapie de précision dans ce sous-type d'ACP et faisons des recommandations pour des tests génétiques en reflexe pouvant être facilement appliqués dans la prise en charge de routine de l'ACP. Pour élucider la majorité inexpliquée de cas d'ACP familial, nous avons utilisé le séquençage de nouvelle génération pour investiguer les exomes germinaux de 109 cas d'ACPs provenant de 93 apparentés «à haut risque» et utilisé une approche par filtre pour identifier plusieurs gènes candidats de susceptibilité au ACP qui sont impliqués dans la réparation de l'ADN. Les plus remarquables sont *FAN1*, *NEK1* et *RHNO1*, dans chacun desquels se trouvaient des mutations pathogènes chez 3 apparentés (3,2%) et démontraient une ségrégation avec l'ACP dans 2 apparentés. L'identification de plusieurs gènes candidats à faible prévalence, plutôt que d'un seul gène majeur dans notre grande série de cas, met en évidence l'hétérogénéité probable de l'hérédité de l'ACP. Une survie défavorable a été observée dans les cas d'ACP au stade précoce avec des mutations germinales dans les gènes de réparation de l'ADN, convergeant vers l'hypothèse que ces cas pourraient représenter un sous-type clinique distinct avec des sensibilités sélectives aux traitements médicamenteux. Dans l'ensemble, cette thèse vise à caractériser la contribution des causes génétiques germinales connues et nouvelles de l'ACP et soutient la notion que des sous-types génétiques distincts d'ACP peuvent bénéficier de thérapies ciblées, soulignant les limites de l'approche généralisée du traitement de l'ACP qui est présentement appliquée.

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First and foremost, I would like to thank my supervisor, Dr. George Zogopoulos, for taking a chance on me as his first graduate student and for offering me more opportunities than I can count over these past six years. His work ethic, passion for science, dedication to trainees and compassion for his patients never cease to amaze me. I would not be where I am today without his unwavering support and guidance.

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I humbly dedicate this thesis to the incredibly courageous patients and families who are true warriors in this fight against pancreatic cancer.

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Preface and Contributions of Authors

This thesis is organized in manuscript-based format according to the guidelines and specifications outlined by the Faculty of Graduate and Postdoctoral Studies of McGill University. Chapter 1 provides an overview of the literature, and concludes with the rationale, hypothesis and aims of this thesis. Chapters 2, 3 and 4 are presented as manuscripts, and are published as follows:

Chapter 2: Smith AL, Bascuñana C, Hall A, Salman A, Andrei AZ, Volenik A, Rothenmund H, Ferland D, Lamoussenery D, Kamath AS, Amre R, Caglar D, Gao ZH, Haegert DG, Kanber Y, Michel RP, Omeroglu-Altinel G, Asselah J, Bouganim N, Kavan P, Arena G, Barkun J, Chaudhury P, Gallinger S, Foulkes WD, Omeruglu A, Metrakos P, Zogopoulos G. (2015). **Establishing a clinic-based pancreatic cancer and periampullary tumour research registry in Quebec.** *Current Oncology*, *22*(2), 113–10. http://doi.org/10.3747/co.22.2300

Chapter 3: Smith AL*, Wong C*, Cuggia A, Borgida A, Holter S, Hall A, Connor AA, Bascuñana C, Asselah J, Bouganim N, Poulin V, Jolivet J, Vafiadis P, Le P, Martel G, Lemay F, Beaudoin A, Zand KR, Chaudhury P, Barkun J, Metrakos P, Marcus V, Omeroglu A, Chong G, Akbari MR, Foulkes WD, Gallinger S, Zogopoulos G. (2018). **Reflex testing for germline *BRCA1*, *BRCA2*, *PALB2* and *ATM* mutations in pancreatic cancer: mutation prevalence and clinical outcomes from two Canadian research registries.** *JCO Precision Oncology*, (2), 1-16. https://doi.org/10.1200/PO.17.00098

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Chapter 4: Smith AL, Alirezaie N*, Connor A*, Chan-Seng-Yue M, Grant R, Selander I, Bascuñana C, Borgida A, Hall A, Whelan T, Holter S, McPherson T, Petersen GM, Omeruglu A, Saloustros E, McPherson J, Stein LD, Foulkes WD, Majewski J, Gallinger S[#], Zogopoulos G[#]. (2016). **Candidate DNA repair susceptibility genes**

identified by exome sequencing in high risk pancreatic cancer. *Cancer Letters* 370(2), 302-312. http://doi.org/10.1016/j.canlet.2015.10.030

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Chapters 2, 3 and 4 begin with prefaces, which describe the state of the field at the time in which each study was conducted, reiterate the rationale and highlight the connection between chapters. Finally, Chapter 5 provides a summary of the results, presents updates in the literature, and discusses the translational implications of our findings and future directions in this rapidly evolving field.

Original Contributions

The work presented in this thesis encompasses our investigations of known and novel DNA repair genes in pancreatic cancer predisposition, using the resources of pancreatic cancer research registries. Within Chapter 2, we describe the establishment of the first and only pancreatic cancer research registry in Quebec, demonstrate that a prospective, clinic-based registry can be successfully implemented in a tertiary care center and leads to higher participation rates than population-based registries, and finally, that this registry allows for the collection of rare "high risk" kindreds for the study of pancreatic cancer predisposition. In Chapter 3, we define the prevalence of 4 known pancreatic cancer susceptibility genes that are implicated in DNA repair (*BRCA1, BRCA2, PALB2* and *ATM*) in a large series of consecutive patients with pancreatic cancer, consolidating what has been described in the literature. Our study represented the largest series of incident pancreatic cancer cases to date evaluating the prevalence of *ATM* mutations, the most recently identified pancreatic cancer predisposition gene. As well, we describe for the first

time the prevalence of French-Canadian founder mutations in *BRCA1*, *BRCA2* and *PALB2* in pancreatic cancer. Further, we provide retrospective clinical outcome data that supports the emerging notion that pancreatic cancer associated with germline mutations in these genes represent a clinical subtype that may have improved outcomes when treated with platinum-based therapies. Finally, we propose criteria for reflex genetic testing in incident pancreatic cancer that can be implemented in ambulatory oncology clinics. In Chapter 4, we used a filter-based exome sequencing approach to identify novel candidate DNA repair pancreatic cancer susceptibility genes. We conclude that the remaining fraction of familial pancreatic cancer is genetically heterogeneous, and propose *FAN1, NEK1* and *RHNO1* as novel candidate genes contributing to a fraction of the unexplained familial clustering of pancreatic cancer. Further, our overall survival analyses point to a hypothesis that patients with pancreatic cancer and rare inherited DNA repair gene variants represent a clinical subtype of pancreatic cancer.

This original work resulted in three published first author manuscripts presented as Chapters 2 – 4 in the present thesis. The studies described here were performed under the supervision of Dr. George Zogopoulos.

Detailed Contribution of Authors

The chapters presented in this thesis were written in their entirety by the candidate, with edits and commentary primarily from Dr. George Zogopoulos, as well as from manuscript co-authors. Detailed author contributions for Chapters 2 through 4 are described below.

<u>Chapter 2</u>: Establishing a clinic-based pancreatic cancer and periampullary tumour research registry in Quebec

Alyssa L. Smith contributed to the study concept and design, participated in patient enrolment, biospecimen collection and QPCS database management, led the analysis and interpretation of data, and drafted and revised the manuscript and figures. **Claire Bascuñana** participated in patient enrolment, work up of families and data analysis. **Anita Hall** managed the QPCS biobank. **Ayat Salman**, **Alexandra-Zoe Andrei**, **Alexandra Volenik**, **Heidi Rothenmund**, **Diane Ferland**, **Daphnee Lamoussenery**, **Ashwin S. Kamath**, **Ramila Amre**, **Derin Caglar**, **Zu-Hua Gao**, **David G. Haegert**, **Yonca Kanber**, **René P. Michel**, **Gulbeyaz Omeroglu-Altinel**, **Jamil Asselah**, **Nathaniel Bouganim**, **Petr Kavan**, **Goffredo Arena**, **Jeffrey Barkun**, **Prosanto Chaudhury**, **William D. Foulkes, Atilla Omeruglu** and **Peter Metrakos** contributed to the acquisition of data and biospecimens. **Steven Gallinger** shared the framework of the Ontario Pancreas Cancer Study, which contributed to the design of the QPCS. **George Zogopoulos** contributed to the study concept and design, supervised the project, contributed to the acquisition, analysis and interpretation of data, and edited the manuscript. All authors approved the final manuscript.

Chapter 3: Reflex testing for germline *BRCA1*, *BRCA2*, *PALB2* and *ATM* mutations in pancreatic cancer: mutation prevalence and clinical outcomes from two Canadian research registries.

Alyssa L. Smith contributed to the study design, extracted and prepared DNA from patient samples for genetic analyses, confirmed variants of interest by PCR and Sanger sequencing, worked up families of mutation carriers for segregation analyses, performed loss-of-heterozygosity experiments, performed chart reviews of Montreal patients for analyses of clinical variables and overall survival, carried out statistical analyses, and drafted and revised the

manuscript and figures. **Cavin Wong** assisted in the confirmation of variants of interest by PCR and Sanger sequencing, contributed to French-Canadian founder mutation testing by PCR and Sanger sequencing, provided bioinformatics support for the interpretation of full gene sequencing data, contributed to statistical analyses, contributed to manuscript figures, and edited the manuscript. **Adeline Cuggia** and **Ayelet Borgida** contributed to the acquisition and analysis of data. **Anita Hall** aided in the processing of blood samples for downstream DNA extraction, and in the preparation of samples for French-Canadian founder mutation testing. **Ashton A. Connor** evaluated RNASeq data to evaluate of a variant predicted to affect splicing, provided the Figure S3.2, and critically reviewed the manuscript. **Spring Holter, Claire Bascuñana**, **Jamil Asselah**, **Nathaniel Bouganim**, **Véronique Poulin**, **Jacques Jolivet**, **Petro Vafiadis**, **Philippe Le**, **Guillaume Martel**, **Frédéric Lemay**, **Annie Beaudoin**, **Khashayar Rafatzand**, **Prosanto Chaudhury**, **Jeffrey Barkun** and **Peter Metrakos** contributed to the acquisition of data and biospecimens. **Victoria Marcus** and **Atilla Omeroglu** confirmed pathologic diagnoses and reviewed histologic slides for macrodissection. **George Chong** carried out the French-Canadian founder mutation testing for 94 patients included in this study. **Mohammad R. Akbari** performed the full gene sequencing and multiplex ligation probe amplification, and provided bioinformatics support. **William D. Foulkes** provided expertise in the interpretation of data and critically reviewed the manuscript. **Steven Gallinger** contributed to the study design and reviewed the manuscript. **George Zogopoulos** guided the study concept and design, acquisition, analysis and interpretation of data, and edited the manuscript. All authors approved the final manuscript.

Chapter 4: Candidate DNA repair susceptibility genes identified by exome sequencing in high-risk pancreatic cancer.

Alyssa L. Smith contributed to the study design, extracted DNA from QPCS patient samples, prepared samples for exome sequencing, performed the filter-based analysis of exome sequencing data, reviewed variants of interest in the Integrative Genomics Viewer, validated variants of interest by PCR and Sanger sequencing, worked up families of mutation carriers for segregation analyses, performed loss-of-heterozygosity experiments, performed chart reviews of QPCS patients, carried out statistical analyses, drafted and revised the manuscript and figures. **Najmeh Alirezaie** implemented the bioinformatics pipeline (i.e., alignment and variant calling) for the whole-exome sequencing data and contributed to the analysis and interpretation of data. **Ashton Connor** provided bioinformatics support and contributed to the analysis and interpretation of data. **Michelle Chan-Seng-Yue** provided bioinformatics support for the WGS data included in the validation studies. **Iris Selander** reviewed variants of interest from the validation set in the Integrative Genomics Viewer and assisted with confirmation of variants of interest by PCR and Sanger sequencing in Toronto samples. **Claire Bascuñana** and **Ayelet Borgida** contributed to the acquisition of data and biospecimens, and assisted with chart reviews. **Thomas Whelan** and **Treasa McPherson** assisted with the confirmation of variants of interest by PCR and Sanger sequencing in Toronto samples. **Robert Grant**, **Anita Hall**, **Spring Holter**, **Sean Cleary**, **Gloria M. Petersen**, **Emmanouil Saloustros**, **John McPherson**, **Lincoln D. Stein**, **William D. Foulkes, Steven Gallinger and George Zogopoulos** contributed to the acquisition of data and biospecimens. **Atilla Omeroglu** reviewed histologic slides for macrodissection. **Jacek Majewski** provided bioinformatics support for the exome sequencing data included in the discovery set, provided access to in-house control exome data, and contributed to the analysis and interpretation of data. **Steven Gallinger** contributed to the study design, analysis and interpretation of data, and reviewed the manuscript. **George Zogopoulos** guided the study concept and design, acquisition,

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List of Abbreviations

Abbreviation	Definition
2DR	Second-degree relative; see also SDR
3DR	Third-degree relative
5-FU	5-fluorouracil
AJ	Ashkenazi Jewish
BER	Base excision repair
BMI	Body Mass Index
BWA	Burrows-Wheeler Alignment tool
CA19-9	Carbohydrate antigen 19-9
CCDS	Consensus Coding Sequence
CI	Confidence Interval
CR	Complete response, as per RECIST 1.1 criteria
CT	Computed tomography
DDR	DNA damage response
DNA	Deoxyribonucleic acid
DSB	Double-stranded break
DSBR	Double-strand break repair
ERCP	Endoscopic retrograde cholangiopancreatography
EUS	Endoscopic ultrasound
EVS	Exome Variant Server
EWAS	Exome-wide association study
ExAC	Exome Aggregation Consortium
FA	Fanconi anemia
FAMMM	Familial atypical multiple mole melanoma
FAP	Familial adenomatous polyposis
FC	French-Canadian
FDR	First-degree relative
FFPE	Formalin-fixed, paraffin-embedded
FPC	Familial pancreatic cancer; see also, fPDAC

fPDAC	Familial pancreatic ductal adenocarcinoma; see also, FPC
FNA	Fine-needle aspiration
FOLFIRINOX	Chemotherapeutic regimen comprising folinic acid, 5-FU, irinotecan and oxaliplatin
GATK	Genome Analysis Tool Kit
GWAS	Genome-wide association study
HBC	Hereditary breast cancer
HBOC	Hereditary breast and ovarian cancer
HDR	Homology-directed DNA repair
HGVS	Human Genome Variation Server
HNPCC	Hereditary non-polyposis colorectal cancer, also known as Lynch syndrome
HPB	Hepatopancreatobiliary
HP	Hereditary pancreatitis
HPC	Hereditary pancreatic cancer
HR	Homologous recombination
ICL	Interstrand crosslink
IGV	Integrative Genomics Viewer
IHC	Immunohistochemical
Indel	Insertion/deletion
IPMN	Intraductal papillary mucinous neoplasm
LCM	Laser capture microdissection
LFS	Li-Fraumeni syndrome
LOH	Loss of heterozygosity
MAF	Minor allele frequency
MCN	Mucinous cystic neoplasm
MLPA	Multiplex ligation-dependent probe amplification
MMR	Mismatch repair
MR	Mixed response
MRI	Magnetic resonance imaging
MT	Montreal-Toronto

MUHC	McGill University Health Centre
NCCN	National Comprehensive Cancer Network
NER	Nucleoside excision repair
NGS	Next-generation sequencing
NHEJ	Non-homologous end-joining
OPCS	Ontario Pancreas Cancer Study
OR	Odds ratio
OS	Overall survival
PAC	Pancreatic adenocarcinoma; see also PC
PC	Pancreatic adenocarcinoma; see also PAC
PDAC	Pancreatic ductal adenocarcinoma
PanIN	Pancreatic intraepithelial neoplasm
PARP	Poly(ADP-ribose) polymerase
PAT	Periampullary tumour
PCR	Polymerase chain reaction
PD	Progressive disease
PDAC	Pancreatic ductal adenocarcinoma
PDX	Patient-derived xenograft
PET	Positron emission tomography
PHQ	Personal History Questionnaire
PJS	Peutz-Jeghers syndrome
PNET	Pancreatic neuroendocrine tumour
PR	Partial response (>30%), as per RECIST 1.1 criteria
PTV	Protein-truncating variant
PV	Portal vein
QPCS	Quebec Pancreas Cancer Study
R	Response (<30%), as per RECIST 1.1 criteria
RFA	Radiofrequency ablation
RNA	Ribonucleic acid
RR	Relative risk
SD	Stable disease, as per RECIST 1.1 criteria; or, standard deviation

SDR	Second-degree relative; see also 2DR
SIR	Standardized incidence ratio
SMA	Superior mesenteric artery
SMV	Superior mesenteric vein
SNV	Single nucleotide variant
SPN	Solid pseudopapillary neoplasm
ss	Single-stranded
SSB	Single-stranded break
TNM	Tumour-node-metastasis
VUS	Variant of unknown significance
WES	Whole exome sequencing
WGS	Whole genome sequencing

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Chapter 4:

Chapter 1: Introduction

1.1 Pancreatic Adenocarcinoma

1.1.1 Anatomy and Histology of the Pancreas

The human pancreas is a glandular organ that lies deep in the abdomen and carries out both exocrine (digestive) and endocrine (metabolic) functions. The exocrine cells of the pancreas, comprising 95% of the pancreatic mass, include the acini which produce and secrete digestive enzymes and the ductal systems which carry these secretions to the main pancreatic duct, ultimately emptying into the duodenum where they function to break down carbohydrates, proteins and lipids.[1] The endocrine cells of the pancreas are found within the Islets of Langerhans, scattered within the exocrine tissue and composed of α, β, δ and PP cells, which secrete blood glucose-regulating hormones – glucagon, insulin, somatostatin, and pancreatic polypeptide, respectively, into the bloodstream.[1,2]

Anatomically, the human pancreas is a long, tapered organ located deep in the abdominal cavity, in the retroperitoneal space, at the level of the L1-L2 vertebrae.[3] It is divided into four anatomical regions, from proximal to distal: head, neck, body, and tail.[3] The head of the pancreas is surrounded by the C-shaped curve of the duodenum, to the right of the superior mesenteric artery (SMA) and vein (SMV), and resting posteriorly on the inferior vena cava.[3] The uncinate process, a projection arising from the inferior part of the head of the pancreas, extends medially towards the SMA and lies posterior to the SMV.[3] The neck of the pancreas is a short segment that overlies the SMA and SMV, which form a groove on its posterior surface.[3] The SMV joins the splenic vein posterior to the neck of the pancreas, forming the portal vein (PV).[3] The body of the pancreas lies posteriorly to the distal portion of the stomach and the tail of the pancreas lies anterior to the left kidney, extending to the hilum of the spleen.[3] The main pancreatic duct begins in the tail of the

pancreas and runs along the length of the gland where it meets the common bile duct in the head of the pancreas, together forming the ampulla of Vater, which joins the duodenum.[2,3]

The proximity and relationship of the structures surrounding the pancreas pose a challenge in the diagnosis and surgical management of pancreatic neoplasms. In particular, since the ampulla of Vater serves as the confluence of the common bile duct, pancreatic duct, duodenum and head of the pancreas, adenocarcinomas arising from these peri-ampullary structures can be difficult to discern from pancreatic adenocarcinoma (PAC).[4] Further, the intimacy of the pancreas with major blood vessels can limit opportunities for surgical resection of pancreatic neoplasms.[5]

1.1.2 Neoplasms of the Pancreas

Neoplasms of the pancreas can arise from either the exocrine or endocrine cells of the pancreas, and can be broadly divided into solid and cystic types.[5] When one refers to "pancreatic cancer", they are typically referring to the most common solid neoplasm of the pancreas, **pancreatic adenocarcinoma** (PAC), which accounts for approximately 85% of all solid pancreatic neoplasms.[6] PAC form glands that acquire the ability to infiltrate into tissues, nerves and lymphatics.[5] An important histological feature of PAC is the extensive stromal desmoplastic reaction that commonly occurs, consisting of fibroblasts, inflammatory cells, endothelial cells and extracellular matrix componenents.[5] Most PAC arise in the ductal cells, termed pancreatic ductal adenocarcinoma (PDAC). Rare variants of PAC include acinar cell carcinoma, adenosquamous carcinoma, colloid carcinoma, medullary carcinoma, signet ring cell carcinoma, undifferentiated carcinomas and undifferentiated carcinomas with osteoclast-like giant cells.[5] Small microscopic lesions called **pancreatic intraepithelial neoplasms** (PanIN) are thought to be a precursor to PAC.[5] These lesions progress from PanIN1 to PanIN3, with increasing degrees of ductal dysplasia,

but contained within the basement membrane (i.e., non-invasive).[7] PanIN3 is also known as carcinoma *in situ*.

Pancreatic neuroendocrine tumour (PNET) is the next most common solid neoplasm of the pancreas, accounting for 5% to 10%. PNETs can be either functional or non-functional tumours, where functional tumours secrete high levels of hormones into the bloodstream resulting in a clinical syndrome (e.g., insulinomas, glucagonomas, and so on).[5]

Additional, very rare types of solid neoplasms of the pancreas include **pancreatoblastoma** and primary **lymphoma** of the pancreas.[5,8]

Most cystic neoplasms of the pancreas are benign, however some have the potential to progress to invasive carcinoma if left untreated. Cystic neoplasms of the pancreas are being more commonly detected as incidental findings with the increasing use and sensitivity of diagnostic imaging studies.[5,8] **Serous cystadenomas** are nearly always benign and are typically followed clinically without need for surgery unless large or symptomatic.[5] **Mucinous cystic neoplasms** (MCNs) and **intraductal papillary mucinous neoplasms** (IPMNs) are noninvasive mucin-producing neoplasms with malignant potential.[6,9] IPMNs, by definition, involve the larger pancreatic ducts and can be classified as main duct or side branch IPMN.[5,9] Main duct IPMNs are associated with higher rates of malignancy.[9] MCNs typically arise in the tail of the pancreas without communication with the pancreatic duct system.[5,9] They most commonly affect females in the fifth decade of life.[8] Finally, **solid pseudopapillary neoplasms** (SPNs) are low-grade malignant neoplasms that arise almost exclusively in younger women and are treated surgically.[5]

In addition to these neoplasms that originate in the pancreas, the pancreas can serve as a site for distant metastases, although uncommonly.[8] Nearly any tumour type may metastasize to the pancreas, however the most common are carcinomas of the kidney, colon and lung.[8,10] When these

metastases present as an isolated pancreatic mass, they may be clinically suspected to be primary pancreatic neoplasms.[8]

The focus of this dissertation is pancreatic adenocarcinoma (PAC), and the terminology "PAC" and "pancreatic cancer" will be used synonymously.

1.1.3 Epidemiologic and Clinical Overview of Pancreatic Cancer

Despite decades of research, surgical advances and modern therapeutic regimens, PAC remains a devastating cancer diagnosis. In 2017, it is estimated that 5,500 Canadians will be diagnosed with PAC and about 4,800 will die from the disease.[11] Among North Americans, the estimated lifetime risk of developing PAC is approximately 1.5%,[12,13] making it only the 12th most common cancer, however its high mortality relative to incidence makes PAC the 4th leading cause of cancer death.[11] It carries the worst 5-year survival rate of any cancer – only 8%.[11,14] As advances in early detection and treatment options continue to lag for PAC relative to other major cancer types, it is projected that PAC will be the 2nd leading cause of cancer death in North America by 2030.[15] These dismal statistics are largely attributable to the advanced stage at diagnosis, precluding curative-intent surgical resection, and chemoresistance of PAC.

Because of the location of the pancreas deep in the abdomen, PAC in its early stages is largely asymptomatic. By the time signs and symptoms present, the PAC is often advanced, having spread beyond the pancreas either locally or to distant sites.[5] Even then, symptoms are often non-specific such as epigastric pain, weight loss, nausea and fatigue.[5,16] Tumours arising in the head of the pancreas, accounting for up to 70% of PAC, can sometimes cause obstruction of the bile duct leading to jaundice, pruritus, clay-colored stools and dark urine.[5,16] Additional signs of PAC can include new-onset diabetes or pancreatitis.[5]

Clinical stage determines the prognosis and treatment for patients with PAC. Staging investigations for PAC might include non-invasive imaging modalities such as computed tomography (CT), magnetic resonance imaging (MRI), and occasionally, positron emission tomography (PET).[5,17] Pathologic confirmation of adenocarcinoma may be obtained by endoscopic retrograde cholangiopancreatography (ERCP)-guided cytologic brushings, or from endoscopic ultrasound (EUS)-guided or percutaneous fine-needle aspiration (FNA) or core biopsies.[5,17] Serum carbohydrate antigen 19-9 (CA19-9) tumour biomarker levels might also be measured and monitored as a surrogate of tumour burden.[17]

The American Joint Committee on Cancer (AJCC) staging system (0, IA, IB, IIA, IIB, III and IV), which incorporates the tumour-node-metastasis (TNM) classification, is most commonly used for staging PAC.[18] More broadly, PAC can be defined in terms of resectability. PAC that has metastasized to distant sites (stage IV), most commonly the liver, peritoneum and lungs, is a precluding factor for curative-intent surgical resection.[5,17] In the absence of metastasis, resectability is largely dictated by the extent of tumour invasion into adjacent major vascular structures (SMA, celiac axis, SMV, PV).[5,17] Stage III PAC can be divided into "borderline resectable" or "locally advanced", where the former has <180-degree involvement (abutment) of the celiac axis or SMA, and the latter, >180-degree involvement (encasement) of those vessels.[5,17] Stages 0-IIB do not have vessel involvement and are considered resectable.[5,17]

Patients with resectable PAC typically undergo upfront *en bloc* tumour resection by either pancreaticoduodenectomy (Whipple procedure), distal, or total pancreatectomy, depending on the location of the tumour,[16] followed by adjuvant chemotherapy with gemcitabine[19] or gemcitabine with capecitabine [oral 5-fluorouracil (5-FU) formulation].[20] Patients with borderline resectable or locally advanced PAC are offered neo-adjuvant chemoradiotherapy, with the goal of downsizing

to resectability.[21,22] Unfortunately, only 20% of all patients are considered candidates for surgical resection.[16]

Between 50 and 60% of PAC is metastatic at the time of diagnosis.[11,14] Metastatic PAC was standardly treated with gemcitabine since 1997.[23] Only in recent years have new cytotoxic combination therapies been approved for metastatic PAC, with significant, albeit marginal (on the order of months), improved survival compared to gemcitabine alone. These include FOLFIRINOX, a regimen comprising folinic acid, 5-FU, irinotecan and oxaliplatin,[24] and nab-paclitaxel (trade name: Abraxane) in combination with gemcitabine.[25] FOLFIRINOX carries significant toxicity and necessitates good performance status.[24]

Even for patients who undergo curative-intent resection and receive adjuvant chemotherapy (gemcitabine in combination with capecitabine), the 5-year survival is only 28.8%.[20] This is in contrast, however, to the less than 3% 5-year survival for patients who present with metastatic disease.[14]

1.1.4 Risk Factors for Pancreatic Cancer

The risk for PAC increases substantially with age, with more than 80% of PAC diagnosed in patients aged 60 years and older.[11] The disease is slightly more common in males than females, likely attributable to differences in exposure to risk factors.[11] Active cigarette smoking is the most important environmental risk factor for PAC, with an estimated 1.5- to 2.2-fold increased risk of PAC for ever-smokers compared to non-smokers, and an estimated population attributable fraction of 11-32%.[26,27] Importantly, this risk is reduced after smoking cessation and eventually reaches the level of never-smokers (after approximately 20 years).[26] Additional risk factors for PAC include: *Helicobacter pylori* infection (relative risk [RR]=1.2-1.7),[27] obesity (RR=1.2-1.6),[27,28] heavy

alcohol consumption (RR=1.1-1.5),[27,29] type 2 diabetes mellitus of more than 10 years duration (RR=1.5-1.7),[30,31] and history of chronic pancreatitis (RR=2.7-5.1).[27,32] Whereas long-standing type 2 diabetes mellitus and chronic pancreatitis are considered risk factors for PAC, new-onset diabetes (<3 years) and new-onset (acute) pancreatitis may be early signs of PAC.[5,33] Protective factors for PAC include history of allergies (RR=0.7-0.8) and increased fruit and folate intake (RR=0.5-1.0).[27]

In addition to these environmental risk factors, important hereditary and genetic factors associated with PAC risk have been described. These include non-O blood type (RR=1.3-1.4),[27,34] a positive family history of PAC, inherited germline mutations in any of 15 known moderate- to high-penetrance PAC susceptibility genes, as well as several common, low-penetrance alleles. The latter three are reviewed in section 1.2 (Genetic Basis of Pancreatic Cancer).

1.1.5 Molecular Pathogenesis of Pancreatic Cancer

Cancer is fundamentally a genetic disease caused by the accumulation of genetic alterations, either inherited in the germline or acquired somatically, of oncogenes and tumour suppressor genes which drive tumorigenesis.[35] Sequencing studies have revealed frequent somatic alterations of 4 main driver genes in PAC.[36] These include activating mutations of the proto-oncogene *KRAS* in >90% of PAC, and inactivating alterations of tumour suppressor genes, *CDKN2A*, *TP53* and *SMAD4* occur at rates of >50%.[36-38] Besides these driver genes, several genes involved in chromatin modification, DNA repair, axon guidance, Wnt signaling, Hedgehog signaling and cell cycle processes have been found to be recurrently mutated in <10% of PAC, however a long list of infrequently mutated genes predominates in PAC, resulting in marked intertumoural genetic heterogeneity.[36-39]

Studies of PAC precursor lesions (i.e., PanINs) have defined the timing of the genetic alterations in pancreatic tumorigenesis and support a classic stepwise progression model from low to high grade dysplasia (PanIN1 to PanIN3) that is associated with the accumulation of genetic alterations.[40] *KRAS* and *CDKN2A* alterations appear to be early events, present in PanINs with low- and intermediate-grade dysplasia,[41,42] whereas *TP53* and *SMAD4* alterations are later events, occurring in high-grade PanINs and invasive PAC.[42,43] A recent whole exome sequencing study showed that a large proportion (>50%) of somatic mutations are shared between PanIN lesions and adjacent PAC in most cases, further supporting the notion that PanINs are precursor lesions which give rise to PAC.[44]

Interestingly, a recent genomics study has challenged this gradual progression model, proposing a new model whereby PAC evolution is accelerated by catastrophic mutational processes such as polyploidization and chromothripsis, resulting in *"en bloc"* inactivation of PAC driver genes (*CDKN2A, TP53, SMAD4*).[45] This model might explain the rapid metastatic potential of PAC and the inability to markedly improve patient outcomes despite efforts aimed at early detection.[45] Evidence in favour of both the classic progression model and the catastrophic progression model suggest that there may be multiple molecular mechanisms that drive pancreatic tumorigenesis.

1.2 Genetic Basis of Pancreatic Cancer

1.2.1 Overview and Definitions of Familial and Hereditary Pancreatic Cancer

The evidence for a genetic basis of PAC come from early case reports of familial aggregation of PAC,[46-57] dozens of observational studies that consistently demonstrate increased risk for PAC in individuals with a family history of the disease (with risk increasing disproportionately as the number of affected relatives increases),[58-79] as well as the inclusion of PAC in the tumour spectrums of several well-defined genetic syndromes. While most cases of PAC are sporadic, familial clustering of PAC is observed in approximately 10% of PAC cases,[65,70] and may be explained by shared genetic factors, common environmental exposures, a combination of the former two, or simply by chance.[80]

The term **"familial pancreatic cancer" (FPC)** is used to describe families with multiple relatives affected with PAC, sometimes strictly defined as families with at least a pair of first-degree (siblings or parent-child) relatives affected with PAC,[81,82] in the absence of a known genetic cause. A germline genetic mutation in any of 15 known PAC susceptibility genes can be found to co-segregate with PAC in up to 15% of these families,[83] collectively termed **"hereditary pancreatic cancer" (HPC)**. These PAC susceptibility genes are implicated in hereditary syndromes whose tumour spectrums often include extrapancreatic malignancies, and are detailed in section 1.2.2 and summarized in Table 1.1. The cause of the remaining approximately 85% of FPC families remains unknown.

In certain genetically homogeneous populations, recurrent "founder" mutations are prevalent in known PAC susceptibility genes, and perhaps in novel FPC/HPC genes. This concept is discussed in section 1.2.3.

It should be noted that familial clustering of PAC or other cancers within their respective tumour spectrums is not a universal feature of HPC, with germline genetic mutations in PAC susceptibility genes reported in seemingly sporadic PAC.[84,85] Conversely, familial clustering of PAC or malignancies within a hereditary cancer tumour spectrum does not necessitate co-segregation of disease-causing variants (i.e., phenocopies).[86] The terms FPC and HPC and therefore not synonymous.

In the present dissertation, I refer to HPC as PAC with an identified genetic cause, while FPC refers to familial clustering of PAC in the absence of a known genetic cause. The term "high-risk" PAC, as mentioned in the proceeding sections, refers to individuals with a PAC diagnosis and a family history suggestive of FPC or HPC, and also includes early-onset PAC cases diagnosed at age 50 years or younger. Further, "sporadic" PAC refers to PAC whose etiology is non-hereditary and "incident" PAC refers to any newly diagnosed PAC that is unselected for genetic risk factors.

1.2.2 Hereditary Syndromes Associated with Pancreatic Cancer

Hereditary pancreatic cancer (HPC) is attributed to inherited germline mutations in any of 15 known PAC susceptibility genes. Table 1.1 summarizes these genes according to their associated hereditary syndromes, which are described herein.

Table 1.1. Summary of hereditary PAC syndromes with causative genes and associated relative and lifetime risks for PAC.

Syndrome	Genes	RR for PAC	Lifetime risk for PAC (to age 70-80)	Cancers within the syndrome tumour spectrum
HBOC	*BRCA2* *BRCA1*	3.5-5.9[87-89] 2.3-3.6[12,91]	Elevated Elevated	Breast, ovary, prostate[90]
HBC	*PALB2* *ATM*	6[92] Elevated	Elevated Elevated	Breast[93] Breast, prostate[94,95]
FAMMM	*CDKN2A*	38[96]	17-58%[97,98]	Melanoma[97]
HNPCC	*MLH1* *MSH2* *MSH6* *PMS2* *EPCAM*	7-9[102,104]	3.7%[104]	Colon, endometrium, ovary, stomach, bile duct, urinary tract, small bowel, brain and skin (sebaceous)[99-103]
FAP	*APC*	4.5[105]	Elevated	Colorectal, thyroid, duodenum, ampulla, hepatoblastomas, medulloblastomas[105-107]
PJS	*STK11/LKB1*	76-132[108-110]	11-36%[108-110]	Colorectal, breast, small bowel, gastric[111]
HP	*PRSS1* *SPINK1* (biallelic)	53-87[112-115]	19-53%[112-115]	None
LFS	*TP53*	7[116]	Elevated	Breast, sarcomas, brain, adrenocortical carcinoma, leukemia, lymphoma, melanoma, lung, gastrointestinal and genitourinary[116-118]
FPC 2 FDRs ≥ 3 FDRs	Unknown	 6.4[66] 32[66]	 8-12%[66,119] 40%[66,119]	Unknown

Abbreviations: RR, relative risk; PAC, pancreatic adenocarcinoma; HBOC, hereditary breast and ovarian cancer; HBC, hereditary breast cancer; FAMMM, familial atypical multiple mole melanoma; HNPCC, hereditary non-polyposis colon cancer; FAP, familial adenomatous polyposis; PJS, Peutz-Jeghers syndrome; HP, hereditary pancreatitis; LFS, Li-Fraumeni syndrome; FPC, familial pancreatic cancer; FDR, first-degree relative. References are indicated in superscript and ranges represent the results from multiple studies.

Hereditary Breast and Ovarian Cancer Syndrome

Hereditary breast and ovarian cancer (HBOC) syndrome is characterized by autosomal dominant inheritance of loss-of-function mutations in the breast cancer type 1 (*BRCA1*) and breast cancer type 2 (*BRCA2*) genes resulting in an increased risk of breast, ovarian, prostate and pancreatic cancers.[90] These two genes encode tumour suppressor proteins involved in homology-directed repair (HDR) of DNA double-stranded breaks (DSBs), a process described in section 1.4.1.

BRCA1 and *BRCA2* are highly penetrant breast and ovarian cancer susceptibility genes. The cumulative lifetime risk to age 80 years among women carrying *BRCA1* mutations is estimated between 65% and 90% for breast cancer, and between 24% and 39% for ovarian cancer.[120,121] Among women carrying germline *BRCA2* mutations, the cumulative lifetime risk to age 80 is estimated between 40% and 45% for breast cancer, and between 8.4% and 10% for ovarian cancer.[120,121]

A role for *BRCA2* in PAC was first suspected after Schutte and colleagues identified a homozygous deletion at 13q12.3 in a pancreatic carcinoma in 1995, a finding which contributed to the cloning of the *BRCA2* gene.[122] Subsequently, germline mutations in *BRCA2* were described in patients with PAC by Goggins and colleagues in 1996, who reported that 7.3% of unselected PAC cases carried germline *BRCA2* mutations.[84] Since then, the mutation prevalence has been shown to be higher in PAC patients from FPC kindreds, from 4% to 17%, making *BRCA2* the most prevalent identifiable cause of hereditary PAC to date.[123-127] Studies have estimated a 3.5- to 5.9-fold increased risk of PAC among *BRCA2* mutation carriers.[87-89]

The risk of PAC in carriers of *BRCA1* mutations is less conclusive, with some studies suggesting a 2.3- to 3.6-fold increased risk of PAC,[12,91] and other studies reporting no association.[89,128,129] Two large studies of PAC patients from FPC families identified *BRCA1*

mutations in 1.1-1.2% of cases, suggesting that *BRCA1* has a role in PAC susceptibility, albeit to a much lesser degree than *BRCA2*.[126,127]

It should be noted that the prevalence of germline *BRCA1* and *BRCA2* mutations may vary among different ethnic populations, particularly among founder populations. While the estimated carrier frequency of *BRCA1* and *BRCA2* mutations in the general population is 1 in 400,[130,131] germline mutations in these genes are 10 times more prevalent among individuals with Ashkenazi Jewish (AJ ancestry).[132,133] The AJ and other founder populations are described in detail in section 1.2.3.

While a family history of cancers of the breast, ovary, prostate or pancreas can be predictive of *BRCA1* and *BRCA2* carrier status in PAC,[126] an unremarkable family history of cancer, that is, seemingly "sporadic" PAC, is not uncommon among mutation carriers.[84,85] Because of this observation, more recent studies have assessed the prevalence of *BRCA1* and *BRCA*2 germline mutations in large series of incident PAC cases. In a clinic-based study of 306 incident PAC cases, Holter and colleagues identified pathogenic germline mutations in *BRCA1* and *BRCA2* in 14 (4.6%) of patients.[134] *BRCA1* mutations accounted for 1.0% of mutation carriers, while *BRCA2* accounted for 3.6%.[134] Excluding patients with AJ ancestry, 10 of 273 (3.7%) patients carried a mutation in either *BRCA1* or *BRCA2*.[134] Notably, the majority of *BRCA1* and *BRCA2* mutation carriers identified in this study did not have a family history typical of HBOC, and more than half of carriers would not have been eligible for genetic testing based on the National Comprehensive Cancer Network (NCCN) *BRCA1* and *BRCA2* genetic testing criteria or the Ontario Ministry of Health and Long-Term Care guidelines.[134] Consistent with this study, Hu and colleagues identified germline *BRCA1* and *BRCA2* mutations in 1 (1.0%) and 2 (2.1%) of 96 PAC patients, respectively, that were unselected for cancer family history.[135]

Hereditary Breast Cancer Syndrome

Two additional genes that increase risk of breast cancer,[93,94] *PALB2* and *ATM*, have also been found to increase risk for PAC. The association of these genes to familial PAC was discovered in recent years with the use of whole exome and whole genome sequencing,[136,137] studies which are discussed in detail in section 1.3. Notably, bi-allelic germline mutations in either of these genes lead to the development of Fanconi anemia.[138,139]

PALB2 encodes partner and localizer of BRCA2, a protein that interacts with BRCA1 and BRCA2 and is required for localization of BRCA2 to nuclear DSB repair foci.[140] The initial study by Jones and colleagues, which identified *PALB2* as a PAC susceptibility, identified mutations in 3 of 96 (3.1%) of FPC cases.[137] Except for one European study that observed *PALB2* mutations in 3 of 81 (3.7%) patients with FPC,[141] subsequent studies of FPC families have reported a much lower prevalence of *PALB2* mutations (0% to 1.0%).[126,127,142-145] A Czech study identified 3 distinct *PALB2* mutations among 152 unselected patients with PAC (2.0%), however four North American studies did not identify any *PALB2* mutations among incident PAC cases.[134,135,142,145] The risk of PAC is elevated 6-fold among *PALB2* carriers, according to one study.[92]

Ataxia telangiectasia mutated (*ATM*) encodes a serine/threonine kinase which plays a role in the repair of DSBs and cell cycle checkpoint control.[146] Its role in PAC susceptibility was discovered by Roberts and colleagues, who identified pathogenic germline mutations in *ATM* in 4 of 166 (2.4%) of FPC families.[136] Subsequent large studies of FPC families identified a similar prevalence of 3.2%.[127,147] The prevalence appears similarly high among incident PAC cases, according to one study that observed 4 germline *ATM* mutations (4.2%) among 96 patients.[135] The estimated *ATM* carrier frequency in the general population is as high as 1%.[146]

Familial Atypical Multiple Mole Melanoma Syndrome

Familial atypical multiple mole melanoma (FAMMM) syndrome is an autosomal dominantly inherited syndrome that is characterized by an increased predisposition toward melanocytic and dysplastic nevi, melanoma and PAC.[97] Heterozygous germline mutations in the cyclin-dependent kinase inhibitor 2A (*CDKN2A*) gene account for up to 40% of cases with this syndrome.[148] The *CDKN2A* gene encodes two different proteins, $p16^{INK4a}$ and $p14^{ARF}$.[149] Both proteins are tumour suppressors that act in cell cycle regulation, with $p16^{INK4a}$ preventing progression through the G_1 cell cycle checkpoint and $p14^{ARF}$ acting to stabilize p53.[149] Mutations affecting either or both $p16^{INK4a}$ and $p14^{ARF}$ have been associated with FAMMM, although mutations affecting $p16^{INK4a}$ are much more common.[148]

It is estimated that mutation carriers have a 38-fold increased risk for PAC according to one prospective study.[96] One study estimated the cumulative lifetime risk of PAC for mutation carriers to be 58%, and as high as 93% among smokers.[98] Another study which considered only carriers of the Dutch p16-Leiden mutation estimated a cumulative lifetime risk of PAC of 17%,[97] suggesting that penetrance may vary by mutation.

The reported prevalence of germline *CDKN2A* mutations in PAC varies by population, as well as family history of PAC and melanoma, ranging from 0% to 5.3%.[98,126,127,150] In a large study of 1537 unselected patients with PAC, mutations were identified in only 9 patients (0.6%), however the prevalence increased to 3.3% and 5.3% when considering those who reported a first-degree relative with PAC or melanoma, respectively.[98] In a large multi-center study, *CDKN2A* mutations were identified in 2.5% of cases meeting the definition of FPC (at least a pair of first-degree relatives affected with PAC), and none were observed among cases with a family history of PAC among more distantly related relatives.[126] No mutations were observed in a smaller study of German

FPC families.[150]

CDKN2A mutations may be more prevalent in populations with founder mutations. Founder mutations in *CDKN2A* have been described in the Swedish population (p.R112_L113insR), as well as a 19-bp deletion in exon 2 in the Dutch population (known as the "p16-Leiden" mutation), and these mutations account for the vast majority of mutations in these populations.[148] Notably, an Italian study found *CDKN2A* to be the predominant PAC susceptibility gene in this population, seen more commonly than *BRCA1*, *BRCA2* or *PALB2*.[151] Among 225 unselected PAC cases, 5.7% were found to carry *CDKN2A* mutation, with prevalence ranging from 2.6% among cases without any family history of PAC or melanoma to 17% among families with two such cancer occurrences and 45% among families with three such cancer occurrences.[151] Strikingly, 31% of patients with FPC (ranges from 20% among families with 2 PAC to 50% in families with 3 PAC) were found to be carriers, none of whom had a family history of melanoma.[151] This is in contrast to other studies where *CDKN2A* mutations were not identified in FPC families in the absence of a family history of melanoma.[152,153] The *CDKN2A* Italian founder mutations, p.E27X and p.G101W, were predominant, accounting for 69% of mutations identified in this Italian case series.[151]

No difference in age of PAC diagnosis has been observed for *CDKN2A* mutations carriers compared to non-carriers,[96,98,151] although one study of PAC patients who underwent clinical genetics assessments for suspected hereditary cancer syndromes reported *CDKN2A* mutations in 2 PAC patients diagnosed at ages less than 50 years.[154]

Notably, somatic inactivating mutations in *CDKN2A* are common in up to 95% of sporadic PAC and present in early precursor lesions of the pancreas (PanIN1-3),[155] further supporting the role for *CDKN2A* in the development of PAC.

Peutz-Jeghers Syndrome

Peutz-Jeghers syndrome (PJS) is a rare autosomal dominant tumour syndrome characterized by an increased lifetime risk of malignancy, with up to 93% penetrance, and multiple hamartomatous intestinal polyps and mucocutaneous pigmentation (pigmented macules of the lips, buccal mucosa and digits), with 100% penetrance.[108] The syndrome is caused by heterozygous germline loss-of-function mutations in the serine threonine kinase 11 gene (*STK11*, also known as *LKB1*).[111] Estimates of population prevalence range widely from 1:8,300 to 1:200,000.[111]

Mutation carriers are at an increased risk for numerous malignancies, most commonly colorectal cancer, followed by breast, small bowel, gastric and pancreatic cancers, among others.[111] Individuals with PJS have a 76- to 132-fold increased risk for developing PAC and an estimated cumulative lifetime risk between 11% and 36%.[108-110] Although rare, PJS confers the greatest inherited risk for PAC of any predisposition syndrome.

Hereditary Pancreatitis

Hereditary pancreatitis is a rare inherited form of chronic pancreatitis, which typically manifests as repeated attacks of acute pancreatitis beginning in childhood and often leads to pancreatic insufficiency by early adulthood.[112] It has an estimated population prevalence of 3 in 1,000,000 in Western countries.[156]

Several genes have been associated with hereditary pancreatitis and have different modes of inheritance. Gain of function mutations in the protease, serine 1 (*PRSS1*) gene encoding the cationic trypsinogen protein account for the majority of hereditary pancreatitis, and follow an autosomal dominant mode of inheritance.[157] Mutations in *PRSS1* can result in enhanced trypsin autoactivation or ineffective deactivation of trypsin, resulting in intrapancreatic trypsin activity and injury to the pancreatic parenchyma.[158,159] Loss-of-function mutations in serine protease

inhibitor, Kazal type 1 (*SPINK1*) gene encoding the pancreatic secretory trypsin inhibitor protein, which guards against inappropriate trypsin activity within the pancreatic acinar cells, cause an autosomal recessive form of hereditary pancreatitis.[159,160]

Individuals with hereditary pancreatitis have a 53- to 87-fold increased risk of developing PAC and a cumulative lifetime risk of PAC of 19% to 53%.[112-115] The risk was found to be approximately doubled for individuals with hereditary pancreatitis who are smokers, who were strikingly found to develop the disease 20 years before non-smokers.[113]

Notably, hereditary pancreatitis is the only hereditary syndrome associated with PAC whose risk for malignancy is confined the pancreas.[112] A challenge with surveillance of HP patients for PAC is the gross calcification and fibrosis of the pancreatic parenchyma resulting from chronic pancreatitis.[161] Prophylactic total pancreatectomy is an option chosen by few patients as there is significant morbidity and mortality associated with this procedure.[162]

Other genes have been associated with hereditary pancreatitis, namely *CTRC*, *CASR*, *CPA1*, and *CFTR*, however an associated risk with PAC has not been well described.[163-165]

Hereditary Non-Polyposis Colon Cancer (or Lynch) Syndrome

Hereditary Non-Polyposis Colon Cancer (HNPCC), also known as Lynch, syndrome is an autosomal dominant hereditary cancer syndrome caused by germline mutations in any of four mismatch repair (MMR) genes – *MLH1*, *MSH2*, *MSH6* and *PMS2*, or by germline deletions of *EPCAM*.[99] Deletions which include the transcription termination site of *EPCAM* lead to abnormal transcriptional elongation from *EPCAM* into *MSH2*, which lies adjacent to *EPCAM* in the genome, resulting in abnormal methylation and silencing of *MSH2*.[99] The MMR repair pathway is described in section 1.4.1.

Individuals with Lynch syndrome carry an elevated lifetime risk of developing colon (69% for men and 52% for women)[103] and endometrial cancers (28% to 54%),[101,103] and to a lesser extent, cancers of the pancreas, ovary, stomach, bile duct, urinary tract, small bowel, brain and skin (sebaceous).[99-102]

Risk estimates for PAC in Lynch syndrome have varied. A large study by Kastrinos and colleagues reported a family history of PAC in 21% of Lynch syndrome families, and estimated that mutation carriers carry a 3.7% cumulative lifetime risk of PAC – a 9-fold increased risk compared to the general population.[104] Geary and colleagues reported a similar 7-fold increased risk of PAC in Lynch families,[102] however Barrows and colleagues reported no significant increased risk.[101]

The reported prevalence of germline mutations in these genes among incident PAC cases is low (0-2%),[135,142] however studies assessing MMR-deficiency in PAC tumours by immunohistochemical (IHC) staining for these proteins or by microsatellite instability testing have shown deficiency of one or more of the four MMR proteins in ~15% of cases.[166-170] Notably, PAC associated with Lynch syndrome often exhibit a characteristic medullary histopathology.[170,171]

Familial Adenomatous Polyposis

Familial adenomatous polyposis (FAP) syndrome is an autosomal dominant disease that is characterized by the development of hundreds to thousands of colonic adenomatous polyps and the development of colorectal carcinoma by the age of 40 with 100% penetrance.[106] FAP can be divided into classic, accounting for 90% of cases, and attenuated (the remaining 10%), which exhibits fewer (<100) colonic adenomas with a more proximal distribution in the colon, and a later age of onset.[172] The penetrance of attenuated FAP is lower than classic FAP, but still carries a considerable lifetime risk of colorectal carcinoma estimated at 69% by age 80.[173] Both classic and

attenuated FAP are caused by heterozygous germline inactivating mutations in the adenomatous polyposis coli (*APC*) gene, and the cause for the differing phenotypes is unclear.[172] The incidence of FAP in the population is estimated between 1:7,000 and 1:24,000.[106]

Prophylactic colectomy by early adulthood has improved survival for individuals with FAP,[174] however there remains a risk of extra-colonic malignancies, including cancers of the thyroid, pancreas, duodenum and ampulla, as well as hepatoblastomas and medulloblastomas.[105-107] One study found that individuals with FAP carry a 4.5-fold increased risk of developing PAC.[105]

Li-Fraumeni Syndrome

Li-Fraumeni Syndrome (LFS) is a rare autosomal dominant hereditary cancer syndrome caused by germline heterozygous mutations in the tumour protein 53 (*TP53*) gene, which encodes the cell cycle regulator and tumour suppressor protein, p53.[117] The syndrome is characterized by multi-organ cancer development, typically at a young age.[117] About half of *TP53* carriers develop cancer by the age of 30,[118] with a cumulative lifetime risk estimated to be up to 73% in men and nearly 100% in women.[175]

The most common malignancies within the tumour spectrum of LFS include breast cancer, bone and soft tissue sarcomas, brain tumours, adrenocortical carcinoma and leukemia.[116-118] An increased risk for lymphoma and melanoma, as well as lung, gastrointestinal and genitourinary cancers, has also been reported.[116-118] One study estimated the risk of PAC to be increased 7-fold in individuals with LFS.[116]

Notably *TP53* is among the most commonly somatically inactivated genes in many cancers,[176] including up to 75% of PAC tumours.[37]

1.2.3 Founder Mutations in Hereditary Pancreatic Cancer

1.2.3.1 The Founder Effect

The founder effect is a genetic phenomenon that occurs when a new (founder) population is established from a very small number of individuals from a larger (parent) population, or when a parent population suffers a dramatic reduction or bottleneck, resulting in an overall loss of genetic variation.[177] If this new population expands in isolation (e.g., geographic or cultural), any disease-associated mutations present in the founding population or arising *de novo* in early generations are likely to be present at higher frequencies compared to more genetically diverse populations.[177]

From a cancer genetics standpoint, there is great interest in studying founder populations for several reasons. Firstly, inherited diseases, including inherited cancer syndromes, are often more prevalent in these populations.[177] Second, the reduced allelic variability and haplotype complexity of these populations increases the power for gene discovery studies.[177-180] As well, founder mutations allow for penetrance analyses in a relatively homogeneous genetic background, allowing for more reliable mutation-associated cancer risk estimates in these populations.[177] Finally, because the spectrum of disease-causing alleles is limited and highly recurrent in these populations, carrier detection and genetic counselling for known genetic syndromes is simplified.[181]

Dozens of founder populations have been described and have contributed to our understanding of monogenic disorders.[177,179,180] Among two of the most studied and well-defined are the **French-Canadian** (FC) population of Quebec and the **Ashkenazi Jewish** (AJ) population. These will be reviewed here in the context of their association with known PAC susceptibility genes (*BRCA1, BRCA2* and *PALB2*).

1.2.3.2 The French-Canadian Population

The >6 million FCs of the province of Quebec, Canada, are descendants of approximately 8,500 permanent French settlers, including only 1,600 women, who colonized Nouvelle France, along the Saint Lawrence River, between 1608 and 1759.[180,182] It is estimated that 2,600 settlers who arrived in Nouvelle-France before 1680 contributed approximately two thirds of the current FC gene pool.[182] After the British conquest in 1759, French immigration ended and the FC population expanded rapidly in relative genetic isolation as a result of linguistic and religious barriers.[182] As a result of these founder effects, over 30 Mendelian disorders have been described in FCs, including Tay-Sachs disease, Fragile X syndrome, and cystic fibrosis, to name a few.[180,182] Interregional migrations within Quebec resulted in regional founder effects that is reflected in demographical clustering of genetic diseases.[180,182] One such region, Charlevoix-Saguenay-Lac-St-Jean, has an especially high prevalence of rare autosomal recessive diseases.[180,182] Notably, in the late nineteenth and early twentieth centuries, between 500,000 and 900,000 FCs emigrated to the United States, particularly the New England States, contributing to the now estimated >2.3 million Americans with reported FC ancestry.[182,183] It should also be noted that the FC population of Quebec is genetically distinct from the Francophone populations of Maritime Canada, known as the Acadians.[182] The Acadian population are descendants of French settlers who populated New Brunswick and Nova Scotia, formerly "Acadia".[182]

Soon after the cloning of *BRCA1* and *BRCA2* in HBOC families, came reports of recurrent mutations in these genes in families with FC ancestry.[184-186] Since then, a total of 19 FC founder mutations, 11 in *BRCA1* and 8 in *BRCA2*, have been described.[184-201] Haplotype analyses of unrelated mutation-positive families have suggested that these mutations indeed arose from common ancestors.[186,190,191,193] The majority of these mutations are truncating mutations, with the

exception of one missense mutation in *BRCA2* (p.Glu3002Lys) whose pathogenicity has been confirmed by functional assays.[202,203] Among these mutations, six have been shown to account for a significant majority of mutation-positive families (*BRCA1*: c.2834_2836delGTAinsC, c.4327C>T and *BRCA2*: c.3170_3174delAGAAA, c.5857G>T, c.8537_8538delAG, c.9004G>A).[196,200,201] In 2007, soon after *PALB2* was newly identified as a breast cancer susceptibility gene, Foulkes and colleagues reported a FC founder mutation in *PALB2* present in in approximately 0.5% of unselected FC women with early-onset breast cancer.[204] These 20 FC founder mutations in *BRCA1*, *BRCA2* and *PALB2* are detailed in Table S3.1.

Numerous studies have assessed the contribution of these mutations to HBOC among FC women, leading to estimates that FC founder mutations underlie approximately 40% of cancers in families with three or more cases of breast and/or ovarian cancer, 16% of unselected ovarian/fallopian tube/primary peritoneal cancers, and 6% of young onset (<50 years of age) breast cancers in the FC population.[195,196,198,201] While a family history of PAC has been reported in carriers of FC founder mutations,[185,186,189,193,200] the contribution of these FC founder mutations to PAC among FCs has not been investigated. It is noteworthy that in 1991, Ghadirian and colleagues reported that among Francophone Montrealers with a diagnosis of PAC, 8% reported a family history of the disease, which may have been in part due to founder mutations in these known PAC susceptibility genes.[58]

1.2.3.3 The Ashkenazi Jewish Population

Perhaps the most extensively characterized *BRCA1* and *BRCA2* founder mutations are found in the AJ population. The "Ashkenazi" Jews are Jews of Eastern and Central European descent who, today, encompass about 10 million people worldwide and represent the largest

genetic isolate in the United States (~6 million).[205] AJs are a population known to have high carrier rates (1 in 4 to 1 in 5) for one of more than 20 known recessive disease mutations (e.g., Tay-Sachs disease).[206] Genetic evidence suggests that the high prevalence of these recurrent mutations in AJs reflect a founder effect resulting from a narrow population bottleneck of approximately 350 individuals within the last millennium, followed by rapid endogamous population growth.[207]

Two founder mutations in *BRCA1* (185delG and 5382insC) and one founder mutation in *BRCA2* (6174delT) have been described in the AJ population, with a combined carrier frequency of 1 in 40.[132,133] This is in contrast to the carrier frequency of 1 in 400 in the general population.[130,131] Among AJ women, these 3 founder mutations account for 7% to 12% of incident breast cancers,[208,209] 30% of breast cancers diagnosed before age 40,[210] 59% of families with two or more women affected with breast or ovarian cancer,[211] and 25% to 62% of unselected ovarian cancers. [210-213] The prevalence of these three mutations among incident PAC cases is between 5.5% to 12.1%.[129,134] The *BRCA2* 6174delT mutation in particular is associated with a 8-fold increased risk of PAC.[85] Non-founder mutations in *BRCA1* and *BRCA2* are rare among AJs.[214,215]

While the FC and AJ founder mutations likely contribute the significant majority of founder mutations in *BRCA1* and *BRCA2* in Canada, several other populations have reported founder mutations in *BRCA1* and *BRCA2*,[216,217] including the Icelandic,[218] Polish,[219] German,[220] Czech,[221] and Greek[222] populations, to name only a few.

1.2.4 Familial Pancreatic Cancer (FPC)

Family history was suspected to be a risk factor for PAC as early as 1973 with case reports of familial aggregation of PAC both across siblings and across generations.[46-57] Since then, familial studies have reported such familial clustering of PAC (two or more affected relatives) in

approximately 10% of PAC cases.[65,223,224] Numerous case-control and cohort studies have attempted to quantify the risk of PAC associated with family history of the disease, and have consistently demonstrated that individuals with a relative affected with PAC are themselves at increased risk of developing PAC. The magnitude of risk, however, has varied substantially between studies, from 1.5- to 57-fold increased risk among individuals with one or more relatives affected with PAC.[58-79] The variability in risk estimates between studies may be due to inaccuracy of self-reported family history data, differences in the degree of relatedness of relatives included in the studies, failure to distinguish between cancers of the exocrine and endocrine pancreas, lack of adjustment for important environmental risk factors (e.g., tobacco consumption), and biases inherent to case-control studies and familial cancer registries (e.g., recall and selection bias).

Case-control studies have generally reported higher risk estimates compared to prospective studies. Large, prospective studies that are less prone to selection bias, and meta-analyses, likely provide the best risk estimates for family history of PAC, and are reviewed in detail below.

In a large Swedish registry-based study that included 10.2 million individuals and 21,000 medically-verified PAC cases, a 73% increased risk for PAC among individuals with an affected parent was observed (SIR = 1.73, 95% CI, 1.13-2.54), after adjustment for age, period, area of residence and socioeconomic status, but not for other important PAC risk factors like smoking status.[63] An Icelandic cancer registry- and genealogy-based study reported a relative risk of 2.3 (95% CI, 1.8-3.0) among first-degree relatives of PAC cases, however adjustments were not made for important PAC risk factors.[79]

An American study of 1.1 million men and women that were followed prospectively over 24 years as part of the Cancer Prevention Study-II (CPS-II) found that a family history in a first-degree relative with PAC carried a 66% increased risk of PAC mortality (multivariate-adjusted

RR = 1.66, 95% CI, 1.43-1.94).[78] Importantly, adjustments were made for important PAC risk factors like age, tobacco consumption, BMI and diabetes.

A meta-analysis by Permuth-Wey and Egan[76] that included 7 case-control studies,[58-61,65,70,225] one prospective cohort study,[62] and one nested case-control study within a prospective cohort,[74] totaling 6,568 PAC cases, calculated an 80% increased risk for PAC among individuals with a family history of PAC (RR = 1.80, 95% CI, 1.48-2.12). The studies included evaluated family history of PAC in any relative (that is, first-, second-, or unspecified-degree). The investigators excluded studies where the comparison group was obtained from a family-based study or registry, in an attempt to minimize selection bias.

Similar results were observed in a large multi-center pooled analysis including data from 1 case-control study and 10 cohort studies from Europe, China and the United States, totaling 1,183 PAC cases and 1,205 controls, found that a family history of PAC in a first-degree relative was associated with a 1.76-fold increased risk of PAC (OR = 1.76, 95% CI = 1.19-2.16), which included adjustments for smoking and diabetes.[64]

While most observational studies have considered only first-degree relatives with PAC, or first- and second-degree relatives together, three studies have assessed the risk in second-degree relatives specifically, with relative risk found to be elevated in two studies,[75,225] and approaching significantly elevated risk in a third study.[79] It should be noted, however, that accuracy of family history data in second-degree relatives may be less reliable than that in first-degree relatives.

Two studies have shown that the risk of PAC increases as the number of affected relatives increases, providing further support for a genetic cause underlying familial aggregation of PAC and important information for risk counselling. Klein and colleagues estimated the risk of PAC to increase from 4.5- to 6.4- to 32-fold with one, two, and three first-degree relatives affected with

PAC, respectively.[66] Jacobs and colleagues reported an increase in relative risk for PAC from 1.64 (95% CI, 1.41-1.92) with one affected relative to 2.94 (95% CI, 1.22-7.06) with two or more affected relatives, after adjustment for important non-genetic risk factors.[78]

While the noted excess risk in persons with a family history is compatible with inherited genetic factors, it may also reflect shared environmental exposures in families. Failure to adjust for these non-genetic factors could overestimate the association between family history and PAC. Several of these studies have adjusted risk estimates for known environmental risk factors,[60-62,65,78,225] including tobacco consumption, which is the most important risk factor for PAC and has been shown to double the risk for PAC,[62] as well as age, alcohol consumption, diabetes and pancreatitis. In all instances, family history of PAC remained an important independent risk factor after multivariate adjustment. Not surprisingly, risk was shown to be greater among individuals with a family history of PAC and a personal history of smoking.[65,77,225,226]

Additional genetic evidence for PAC comes from a large twin study by Lichtenstein and colleagues in 2010 who reported a higher concordance for PAC among monozygotic (who share all germline genetic variants) than dizygotic twins (who share approximately 50% of germline genetic variants) ($P = 0.03$), suggesting that familial clustering of PAC is more likely due to shared genetic effects than shared environmental effects.[227]

Finally, a family history of PAC has been associated with elevated risk for cancers of the liver,[77,228] gallbladder,[71] bile duct,[228] breast, [228] and ovary, [228] and lymphoma,[229] while a family history of colon,[78,225,226,230] breast,[225,230] ovary,[71,226] prostate,[64] gallbladder,[61] liver,[78] uterus,[230] stomach,[78] lung,[63] and early brain/central nervous system[71] were each found to be associated with an increased risk of PAC. These findings may point to known or undiscovered hereditary syndromes whose tumour spectrums include PAC.

1.2.5 Early-Onset Pancreatic Cancer

Early age of cancer onset is a hallmark feature of most hereditary cancer syndromes.[231] For example, carriers of *BRCA1* and *BRCA2* mutations have been reported to develop breast and ovarian cancers at a younger age than patients with sporadic cancers.[232] In PAC associated with mutations in these same genes, however, some studies have reported younger age of PAC onset,[125,126,185] while many others have found age of diagnosis to be the same among carriers and non-carriers.[84,124,129,134]

In the setting of FPC, some studies have suggested earlier age of onset in FPC cases compared to their sporadic counterparts,[58,67,226,233] however several studies have found no difference in age.[64,76] Another study suggested that relatives of young-onset PAC patients have a higher risk of themselves developing PAC, even in the absence of additional PAC-affected relatives.[69] There are also reports of genetic anticipation in FPC families, meaning that age of onset of PAC is earlier in each successive generation.[234,235]

This variability in age of PAC onset in the setting of known hereditary cancer syndromes and FPC might be explained by variable penetrance in individual patients, genotype-phenotype associations, or by the contribution of genetic or environmental risk-modifying cofactors (e.g., smoking). While there is a lack of consensus in the literature as to the importance of early onset of PAC, it seems reasonable to classify these patients as "high risk" for inherited predisposition.

1.2.6 Genetic Risk Prediction Models

To quantify the magnitude of risk of PAC in individuals with a family history of the disease, Wang and colleagues developed a Bayesian prediction model (PancPRO) which takes into account the ages at cancer diagnosis, family size, and the relationship between family members.[236]

PancPRO estimates the probability of carrying a PAC susceptibility gene and the associated lifetime risk of PAC in unaffected individuals with a family history of PAC.[236] The model was validated using data from 6,134 individuals across 961 families enrolled in the National Familial Pancreatic Tumor Registry.[236]

PancPRO builds on the foundation developed in BRCAPRO, a Bayesian prediction model which estimates an individuals' risk of carrying a *BRCA1* or *BRCA2* mutation and their corresponding lifetime risk of developing breast and/or ovarian cancers, based on their cancer family history.[237]

These models can be useful in genetic counselling and risk stratification of individuals for genetic testing and screening programs.

1.2.7 Early Detection Screening for Pancreatic Cancer

Since most patients with PAC present with locally advanced or metastatic disease that precludes curative-intent surgery, one method to improve survival rates might be to implement early detection screening programs. Population-based screening for PAC is not feasible since PAC is rare in the general population and there is currently no reliable, inexpensive and non-invasive screening tool or biomarker available. However, studies have shown that individuals with increased genetic risk for PAC might benefit from an imaging-based screening program.[238-250] In 2012, the International Cancer of the Pancreas Screening (CAPS) Consortium put forth guidelines recommending screening for the following high-risk individuals: (1) individuals with two or more PAC-affected blood relatives, with at least one FDR, (2) individuals with Peutz-Jeghers syndrome, irrespective of family history of PAC, (3) *BRCA2* mutation carriers with one affected FDR or two affected relatives, (4) *CDKN2A*, *PALB2* and MMR gene mutation carriers with at least one affected

FDR.[251] There is no consensus, however, on the optimal screening modality, nor at what age and at what interval screening should be performed.

Current imaging modalities for detecting pancreatic lesions include endoscopic ultrasound (EUS), endoscopic cholangiopancreatography (ERCP), magnetic resonance imaging/magnetic resonance cholangiopancreatography (MRI/MRCP) and computed tomography (CT). Since ERCP is an invasive approach associated with potential morbidities (e.g., perforation, acute pancreatitis) and CT poses increased cancer risk due to radiation exposure (particularly in the setting of genetic predisposition to cancer), neither of these imaging modalities are suitable for use in a screening program.[252,253] A recent study comparing MRI to EUS in identifying pancreatic lesions showed that EUS is particularly sensitive for the detection of small solid lesions, while MRI is more sensitive for the detection of small cystic lesions, suggesting that both EUS and MRI should be considered within a screening setting in order to optimize the detection rate of clinically relevant pancreatic lesions.[248]

The success of a screening program for PAC is largely dependent on the validity of the gradual progression model of PAC pathogenesis.[40] This model is supported by a rapid autopsy study which estimated that PAC develops over a ten year period following the initiating tumour cell mutation,[254] suggesting that there is significant lead-time for screening and the opportunity to detect precursor PAC lesions, as well as early stage and potentially curable PACs. The recently proposed catastrophic model of PAC pathogenesis, however, challenges this model, suggesting that in some PACs, massive genetic events (e.g., copy number changes and extensive chromosomal rearrangements as a result of chromothripsis) occur early in tumorigenesis, resulting in simultaneous inactivation of canonical PAC genetic drivers, rapidly conferring a cell with invasive

and metastatic properties.[45] This model, which warrants further investigation, certainly narrows the supposed window of opportunity for early detection screening strategies.

1.3 Gene Discovery in Familial Pancreatic Cancer

While many PAC susceptibility genes have been identified, the majority (approximately 85%) of FPC remains uncharacterized. Several methods of gene discovery have been employed in an attempt to uncover novel PAC susceptibility loci, including traditional linkage analyses, candidate gene studies, whole-exome and whole-genome sequencing studies, and genome-wide association studies.

Two groups have reported statistical evidence for autosomal dominant inheritance of a major PAC susceptibility gene in FPC. Klein and colleagues conducted a segregation analysis of 3,132 individuals in 287 families with probands affected with PAC, to test whether the pattern of PAC in families was consistent with genetic transmission.[255] The study supported an autosomal dominant inheritance model and estimated that 7 out of 1000 individuals in the population are germline carriers of a high-risk PAC susceptibility allele.[255] A smaller study of 70 FPC families by Banke and colleagues similarly reported evidence for autosomal dominant inheritance of a major PAC risk allele.[256]

Genetic linkage analysis is a statistical method that is used to determine the chromosomal location of disease-causing alleles. Its premise is that genetic loci that reside physically close to one another on a chromosome are likely to remain linked during meiosis (termed "linkage disequilibrium") and therefore inherited as a single unit from parent to offspring.[257] Genetic markers (typically microsatellite markers) are evaluated in affected and unaffected members of a pedigree to examine whether they are inherited jointly with the disease phenotype (e.g., PAC), thereby suggesting that the disease locus is in proximity to the marker locus.[257] The LOD score is the statistical estimate of whether two loci are likely to lie in proximity to each other on a chromosome, with a LOD score of 3 or more typically considered strong evidence in favor of

linkage.[257] Fine-mapping of the region of linkage is then required to identify the specific disease-causing mutation. Several cancer susceptibility genes have been discovered using this approach, including known PAC susceptibility genes: *BRCA1*,[258,259] *BRCA2*[260,261] and *STK11*.[262,263]

In 2002, the first successful FPC genetic linkage study was performed on an exceptional FPC kindred of Western European descent, known as "Family X". The clinical features of Family X members were atypical for either sporadic or familial PAC in that affected family members were diagnosed at a younger age (median age 43 years) and nearly always displayed a prodrome of diabetes and pancreatic insufficiency.[235] A surveillance program involving endoscopic ultrasound and ERCP (the first of its kind) was initiated in this family and identified additional family members with precursor pancreatic lesions (PanIN2 or PanIN3).[238] Overall, 20 family members over 4 generations were affected with either PAC (n=9), precursor lesions (n=9) or displayed prodromic signs (n=2), and the disease followed an autosomal dominant inheritance pattern with high penetrance.[235] Genotyping of 35 family members in Family X revealed significant linkage of PAC to the chromosomal position 4q32-34, a region covering 16 megabases and approximately 250 genes.[235] The same group sought to identify the causal gene in this region using a combination of microarray expression data to search for abnormalities in the expression of genes in this region in PAC and precursor lesions compared to normal pancreatic tissue, as well as sequencing of the germline DNA of Family X members for 20 candidate genes within the region.[264] Overexpression of a gene called Palladin (*PALLD*) was observed in the PanIN lesions and PAC of Family X members. Quantitative RT-PCR experiments identified overexpression of *PALLD* in additional sporadic pancreatic precursor lesions and PAC cases that were evaluated, as well as in the adjacent normal pancreatic tissue of these lesions.[264] Sequencing of candidate genes in the germline DNA of Family X members revealed a missense mutation (p.P239S) in *PALLD* that co-segregated

completely with all affected Family X members, and was absent in all unaffected members.[264] No mutations were identified in the remaining 19 genes that were sequenced. *PALLD* encodes a cytoskeletal scaffold protein, and the P239S mutation lies at an evolutionarily conserved amino acid residue in a region coding for an essential alpha-actinin binding domain.[264] Functional assays revealed that the P239S mutation exhibited abnormal cytoskeletal changes and increased cell motility. It was therefore proposed that *PALLD* is a novel highly-penetrant PAC susceptibility gene, that is overexpressed in both familial and sporadic PAC, and acts as a proto-oncogene.[264]

Subsequent studies, however, have failed to substantiate the role for *PALLD* in PAC predisposition or in PAC pathogenesis. Two independent studies of 77 European and 42 American FPC kindreds failed to replicate linkage to the 4q region.[265,266] Genotyping of the specific P239S mutation did not identify additional mutation carriers in 74 FPC families and 9 young onset PAC cases from Europe.[267] A second study of the P239S variant and surrounding sequence identified the P239S variant in the germline of one PAC case of 51 FPC and 33 young onset cases tested, and 1 of 555 unaffected controls.[268] The affected case was diagnosed with PAC at age 74 and had a FDR with PAC diagnosed also in their seventies, and the control case was 91-years old with no personal or family history of cancer,[268] findings which are inconsistent with the early onset and high penetrance of PAC observed in Family X. Sequencing of the surrounding area identified only a common missense mutation (p.G236S) adjacent to P239S.[268] Further, sequencing of the entire *PALLD* gene in 48 FPC cases did not identify any deleterious mutations.[269] In regards to the role of *PALLD* as an oncogenic driver in PAC, sequencing of the entire PAC genome in 2008 failed to identify any somatic mutations in *PALLD*,[36] a finding that is consistent with more recent whole exome and whole genome sequencing studies of large series of PAC.[37,39] Further, Hruban and colleagues demonstrated by immunohistochemistry labelling that *PALLD* is not expressed at

significant levels in neoplastic cells but rather in the surrounding non-neoplastic stromal cells of PAC,[270] a finding that was unappreciated in the original study by Pogue-Geile and colleagues[264] since their expression analyses were performed on bulk PAC tissues. While these studies do not completely rule out *PALLD* as a susceptibility gene in Family X, they conclude that *PALLD* is neither an oncogenic driver in PAC, nor a major contributor to FPC. The possibility that another gene within the 4q32-24 locus is responsible for the PAC susceptibility in Family X is plausible.

Unfortunately, there have been no other successful linkage analysis studies in FPC. Several features of PAC and FPC hamper the success of this approach. Firstly, the small number of affected individuals in most FPC families and the rapid demise of patients with PAC soon after diagnosis, precluding sample collection, limit the number of affected individuals available for analysis.[82] Second, the penetrance of FPC is largely unknown, however is suspected to be reduced compared to previously mapped cancer susceptibility genes,[82] which limits the power of this approach. Furthermore, linkage analysis loses robustness in the presence of locus heterogeneity (i.e., multiple genes causing the same phenotype), a feature that is not unlikely in FPC given the number of genes (~15) that have been implicated in HPC to date.[82] Finally, the absence of a distinguishing clinical phenotype in FPC compared to sporadic PAC, and the late age of onset of PAC, even in the setting of known or suspected genetic predisposition, introduces the possibility of phenocopies (that is, PAC that develops in a family member who did not inherit the disease-causing mutation), which greatly confound the results of linkage analyses.[82]

Candidate gene studies are a feasible and cost-effective approach for gene discovery. They are, however, inherently biased because they depend on an *a priori* hypothesis about the role of a selected candidate gene(s), or group of pathway-related genes, on the disease of interest.[271] Following the discovery that germline bi-allelic *BRCA2* (*FANCD1*) mutations is a cause of

Fanconi anemia,[272] and given the known role of *BRCA2* in PAC susceptibility, three studies sought to evaluate the contribution of additional members of the Fanconi anemia pathway in PAC predisposition. Van der Heijdan and colleagues identified mutations in *FANCC* and *FANCG* in 4 tumours or cell lines from patients with relatively young-onset PAC,[273] however a subsequent study by Rogers and colleagues of 38 individuals with FPC did not identify germline pathogenic mutations in either of these genes.[274] Couch and colleagues sequenced the *FANCC* and *FANCG* genes in a large series of 421 unselected PAC cases and found 2 truncating mutations in *FANCC* (0.5%) in young-onset patients compared to no truncating mutations observed in 654 unaffected controls.[275] No truncating mutations were identified in *FANCG*.[275] In another study, Rogers and colleagues evaluated 44 FPC patients for mutations in *FANCA*, and identified a known disease-associated variant in one patient, however the variant was subsequently detected at a similar prevalence in additional FPC cases and unaffected controls.[276] Based on these studies, there is insufficient evidence to support a role for *FANCA*, *FANCC* or *FANCG* in PAC predisposition.

High-throughput next-generation sequencing (NGS) technologies overcome many of the limitations associated with linkage analyses, providing greater power for gene discovery with reduced bias compared to candidate gene studies. This technology generates hundreds of megabases of nucleotide sequence reads in a single instrument run, allowing the sequencing of an entire human genome in a short timeframe (~days) and at a reasonable cost (~$1000).[277] Applications of NGS include whole-genome sequencing (WGS), as well as targeted sequencing of specific genomic regions of interest, including whole-exome sequencing (WES) and multi-gene panel sequencing. With NGS, the germline DNA from many individuals from different FPC kindreds can be sequenced, increasing the power to detect disease-causing alleles of moderate penetrance and overcoming the challenges of locus heterogeneity. Many cancer susceptibility

genes have been uncovered using this approach, including two novel PAC susceptibility genes that are reviewed below.

In 2009, the first use of whole-exome sequencing to identify the genetic cause of a hereditary disease lead to the identification of *PALB2* as a PAC susceptibility gene.[137] Jones and colleagues used traditional Sanger sequencing to analyze the coding regions of more than 20,000 genes in both the germline and tumour of a patient with FPC. More than 15,000 variants were identified that were not present in the human reference genome. The group hypothesized that any PAC susceptibility gene would contain a heterozygous loss-of-function mutation in the germline, and a second mutation or loss of heterozygosity of the wild-type allele in that gene in the tumour. Using this filter-based approach, three genes, *SERPINB12*, *RAGE* and *PALB2* were found to meet these criteria. Since *PALB2* had previously been associated with breast cancer susceptibility, and since inactivating mutations in *SERPINB12* and *RAGE*, but not in *PALB2*, were found to be relatively common in healthy individuals, *PALB2* was considered the best candidate. To validate this finding, *PALB2* was then sequenced in an additional 96 FPC cases, and three were found to carry truncating mutations (3.1%). The mutation was found to segregate with PAC in one of the FPC families for which DNA was available from multiple affected family members. Since this discovery, there have been numerous studies evaluating the contribution of *PALB2* to PAC, and these are reviewed in detail in section 1.2.2.

Although the identification of *PALB2* as PAC susceptibility relied on traditional Sanger sequencing to sequence the entire exomes from the germline DNA of a single FPC patient and their corresponding tumour, it serves as a proof of principle for the potential of whole exome sequencing in gene discovery. Since then, improvements in technology and decreasing costs of NGS technologies have allowed for large-scale studies of FPC using this approach.

In 2012, Roberts and colleagues successfully applied NGS technologies to identify *ATM* as a novel PAC susceptibility gene.[136] The authors generated whole-genome sequencing data from 16 PAC-affected individuals from six FPC families, and whole-exome sequencing from 22 patients from 10 FPC families. A filter-based approach was then used to narrow down the sizeable list of detected variants based on assumptions that any causative variant would be rare in the general population, heterozygous in an affected individual, would result in loss-of-function of its encoded protein (e.g., producing a nonsense, frameshift or splice-site mutation), and would be present in all PAC-affected family members within a kindred.[136] Two distinct nonsense mutations in *ATM* were identified in 2 kindreds. To validate this finding, the authors sequenced the entire coding regions of *ATM* in an additional 166 FPC cases and 190 spouse controls. Four mutations were identified in cases and none in controls (Fisher's exact test, $P = 0.046$).[136] Tumour DNA was available from one mutation carrier and LOH of the wild-type allele was observed in *ATM*, further supporting its role as a PAC susceptibility gene. Subsequent studies evaluating the prevalence and contribution of *ATM* to PAC are discussed in section 1.2.2.

Finally, genome-wide association studies (GWAS) are an unbiased statistical method aimed at identifying common germline genetic variants that are associated with increased risk of disease. These studies utilize microarrays to genotype hundreds of thousands to millions of common germline variants (mostly single nucleotide polymorphisms, SNPs) across the genome in large numbers of case and control subjects.[278] Since these studies are aimed at identifying common, low-penetrance susceptibility loci, GWAS studies in PAC are discussed only briefly below since the present dissertation is focused on high-risk alleles for PAC.

Since 2009, several large GWAS studies of sporadic PAC have been conducted and have identified putative common PAC susceptibility loci. GWAS studies of individuals with PAC and

primarily European descent identified associations with the following loci (with associated gene(s) in parentheses): 9q34 (*ABO*),[279,280] 1q32.1 (*NR5A2*),[280,281] 5p15.33 (*CLPTM1L/TERT*),[280-282] 13q22.1 (*KLF5*),[280,281] 7q32.3 (*LINC/PINT*),[280,282] 16q23.1 (*BCAR1/CTRB1/CTRB2*),[280,282] 13q12.2 (*PDX1*),[280,282] 22q12.1 (*ZNRF3*),[280,282] 2p13.3 (*ETAA1*),[280] 3q29 (*TP63*),[280] 7p13 (*SUGCT*)[280] and 17q25.1 (*LINC00673*)[280]. A GWAS of PAC in the Japanese population identified three novel loci with significant association to PAC: 6p25.3 (*FOXQ1*), 12p11.21 (*BICD1*) and 7q36.2 (*DPP6*),[283] and a GWAS conducted in the Chinese population identified an association with the previously reported 13q22.1 loci, as well as five novel PAC susceptibility loci at chromosomes 21q21.3 (*BACH1*), 5p13.1 (*DAB2*), 21q22.3 (*TFF1*), 22q13.32 (*FAM19A5*) and 10q26.11 (*PRLHR*).[284] It should be noted that associated variants identified in GWAS studies may not themselves be causal, but rather in linkage disequilibrium with the true causal variant. Functional and fine-mapping studies are likely necessary to discern the causal variants and their etiology in PAC.

1.4 DNA Repair Deficiency in Pancreatic Cancer

1.4.1 Overview of DNA Repair Pathways and the DNA Damage Response

The repair of DNA damage is a complex cellular process that is reliant on timely and effective recognition of DNA damage (i.e., DNA damage response; DDR) and several DNA repair pathways to remedy the various types of lesions that can occur as a result of endogenous or exogenous genotoxic insults, and is critical for the preservation of genomic integrity and maintenance of health.[285] A healthy cell that has acquired DNA damage beyond its ability to repair will undergo apoptosis (programmed cell death) as a means of protecting the organism as a whole from mutated cells with malignant potential.[176] Cells that proceed through DNA replication and cell division without repairing DNA errors can acquire mutations that drive malignant transformation and cancer progression. It is therefore not surprising that genes encoding proteins involved in the DDR and DNA repair are among the most commonly mutated genes in cancer.[286,287] While these mutations are typically acquired somatically, they may also be inherited in the germline. Indeed, many of the known cancer susceptibility genes (in PAC and other cancer types) are key players in the DDR or DNA repair pathways (e.g., *BRCA1, BRCA2*, mismatch repair genes).

While erroneous DNA repair is often favourable to the cancer cell, allowing for accumulation of cancer-driving genomic changes, it also represents a vulnerability of the cancer cell that can be exploited therapeutically. Excessive DNA damage and the resultant genomic instability will prove lethal in even the most aggressive of cancer cells, which might explain why typically only isolated DNA repair pathways are inactivated in cancer cells.[286] The loss of one or more DDR or DNA repair pathway in cancer cells, leads to a greater dependency on the remaining pathways which may be compensating.[288] Opportunities for targeted therapy include treating the

cancer cells with DNA damaging chemotherapeutic drugs that directly target the pathway at fault, or targeting a remaining intact DNA repair pathway, resulting in a synthetic lethal effect, overloading the cancer cells with DNA damage that is ultimately lethal.[288] Examples of such targeted therapy approaches in PAC are described further in section 1.4.3.

The DDR and DNA repair pathways are extremely complex, with hundreds of proteins having been implicated.[288] The various genotoxic lesions that can arise in a cell are repaired by distinct, but partially overlapping, DNA repair pathways that are described only briefly herein.

Base excision repair (BER) is responsible for the repair of small, non-helix-distorting base modifications that include uracil residues in DNA that are created by the deamination of cytosines and damaged bases caused by reactive oxygen species, hydrolytic reactions and methylation.[289] Additionally, the downstream steps of BER are utilized to repair single-stranded breaks (SSBs).[286] Damaged bases are detected and excised by DNA glycosylases that vary depending on the type of damage, generating an abasic site.[289] This abasic site is then incised at the 5' position by the APE1 endonuclease, resulting in a SSB.[290] SSBs are bound by PARP1, which subsequently parylates itself and other targets, and recruits BER proteins such as DNA-Polβ, which cleaves the 5'-deoxyribosomophosphate residue and fills the gap, and the XRCC1-DNA ligase IIIα complex, which ligates the nick.[289,290]

Bulky, helix-distorting adducts, including those caused by ultraviolet radiation and platinum-based chemotherapies, are repaired by **nucleotide excision repair (NER)**.[286] NER can be broken down into 4 major steps: recognition, incision/excision, re-synthesis and ligation.[286] Recognition occurs two ways: the bulky lesion may be recognized during global genome repair, where NER recruits XPC/RAD23B and RPA/XPA complexes to the damaged site, or during transcription, where NER recruits a complex of XPG and CSB to sites of RNA polymerase

stalling.[286] These processes lead to the recruitment of TFIHH, which incorporates the helicases XPB and XPD and unwinds the DNA helix around the damaged site.[286] The complexes XPF/ERCC1 and XPG are then recruited, whose nuclease activity cuts the DNA at the 5' and 3' ends of the lesion, respectively.[286] This dual incision leads to the removal of an approximate 30 base pair single-stranded (ss) DNA fragment that is then resynthesized by complexes consisting of DNA-Polδ/ε, RFC and PCNA, or alternatively, DNA-Polδ/ε and XRCC1.[286] DNA ligase I or IIIα seal the nicks to complete NER.[286]

Mismatch repair (MMR) is an important post-replicative process that recognizes and removes mismatched bases or small insertion or deletion (indel) loops that were missed by the proofreading activity of DNA polymerases during DNA replication.[286] The lesions are initially detected by the MSH2/MSH6 protein heterodimer, which then recruits the MLH1/PMS2 heterodimer.[286] PCNA is loaded onto the daughter DNA strand near the site of the mismatch and stimulates the endonuclease activity of PMS2, which introduces single-strand nicks on either side of the mismatch.[286] These nicks facilitate the excision of the error-containing segment by exonuclease 1 (EXO1) and the resulting gap is then filled by DNA Polδ.[289] Misinserted bases are a common occurrence in short repetitive regions of the genome, like microsatellite repeat regions, and therefore defects in the MMR pathway results in a high mutation load in these regions, termed "microsatellite instability".[289]

Double-strand breaks (DSB) are generally considered to be the most toxic of all DNA lesions since faulty repair can lead to gross deletions and chromosomal rearrangements.[291] Mammalian cells employ two distinct pathways for DSB repair – **homologous recombination (HR)** and **non-homologous end-joining (NHEJ)**, which are used preferentially based on the presence or absence of a homologous sister chromatid, respectively. HR is therefore restricted to

the post-replicative S- and G_2- phases of the cell cycle, when an identical sister chromatid is available as a template for repair, and is considered to be an error-free repair mechanism.[286] Once HR is initiated, the DSB is resected by nucleases, including MRE11, as part of the MRE11-RAD50-NBS1 (MRN) complex, and CtBP-interacting protein (CtIP), generating a ssDNA overhang.[292] The ends are resected further by exonucleases EXO1 and BLM-DNA2, producing long stretches of ssDNA that are rapidly coated by replication protein A (RPA).[292] RPA is replaced by RAD51 with the help of mediator proteins that include RAD52, BRCA2 and RAD51 paralogs (RAD51B, RAD51C, RAD51D, XRCC2 and XRCC3).[292] The resulting RAD51-ssDNA nucleoprotein filament invades the homologous strand, which is used as a template for DNA synthesis of the resected strand.[292]

NHEJ is another mechanism of DSB repair. NHEJ is not reliant on an intact template and is therefore preferentially activated during the pre-replicative G_1-phase of the cell cycle.[286] When NHEJ is activated, the free ends of DSBs are detected and bound by the Ku70/Ku80 heterodimer.[286] This complex recruits DNA-PK_{cs}, whose kinase activity triggers recruitment and activation of end-processing factors, such as Artemis, that trim the DNA ends, making them compatible for ligation.[285] Ligation of the DSB is then executed by the Ligase IV/XRCC4/XLF complex.[285] To guide repair, NHEJ uses short homologous DNA sequences, called microhomologies, which are often present in the single-stranded overhangs on the ends of DBSs.[286] Unless the overhangs are perfectly compatibly, strand resection and annealing of short areas of homology is often required, resulting in nucleotide deletions.[286] For this reason, NHEJ is considered an error-prone mechanism of DSB repair.[286]

The repair of interstrand crosslinks (ICLs) requires the cooperation of the Fanconi anemia (FA), NER and HR proteins.[293] ICLs result from endogenous metabolites and are also induced by

Mitomycin C and platinum-containing chemotherapeutic agents.[293] The **Fanconi anemia (FA) pathway** is so-named because inherited biallelic inactivating mutations of any of the 19 FA proteins results in Fanconi anemia, a rare genetic instability disorder that is characterized by bone marrow failure, developmental abnormalities and predisposition to cancer.[293] The proteins involved in the FA pathway can be grouped into three functional subgroups: the FA core complex, the FANCD2/FANCI complex, and FA effector proteins.[286] Stalled replication forks on DNA ICLs are first recognized by the FANCM/FAAP24/MHF1/MHF2 complex which recruits the FA core complex comprising eight proteins (FANCA, B, C, E, F G, L, M).[286,293] Following assembly, the protein core complex activates FANCL and its partner UBE2T, resulting in monoubiquitination of the FANCD2-FANCI complex.[286,293] The FANCD2-FANCI complex facilitates ICL repair through the downstream effector proteins: FANCD1 (BRCA2), FANCJ (BRIP1/BACH1), FANCN (PALB2), FANCO (SLX4) and FANCP (RAD51C).[286] The nucleases FAN1 and SLX4 are also recruited to the mono-ubiquitinated FANCD2-FANCI complex where they initiate nucleolytic cleavage.[286] Subsequently, MUS81/EME1 and the NER nucleases XPF/ERCC1 mediate further cleavage, facilitating ICL unhooking.[286] This results in formation of a DSB at the site of the stalled replication fork, which is then repaired by HR.[286]

These DNA repair pathways are all influenced by the larger **DNA damage response (DDR)** that regulates the intra-cellular signaling events and enzyme activities that are required to detect DNA lesions, induce cell-cycle arrest, DNA repair or apoptosis following DNA damage.[285] The initial response to DNA damage includes activation of effector kinases, such as ATM and ATR, which are recruited to and activated by DSBs and RPA-coated ssDNA, respectively.[294] ATM and ATR phosphorylate downstream substrates, including checkpoint kinase proteins CHK1 and CHK2, and tumour suppressor protein p53, which act to slow down or arrest cell-cycle progression

at the G_1-S, intra-S and G_2-M "cell cycle checkpoints", allowing more time for repair of the DNA damage before replication or mitosis resumes. ATM/ATR signaling also recruits and promotes activation of repair proteins to the site of damage.[294] If DNA damage cannot be adequately repaired, chronic DDR signaling induces p53-mediated apoptosis or cellular senescence.[294] P53 is a key player in the tumour suppressive DDR and is inactivated in approximately 50% of human cancers, including PAC.[176] As previously discussed, germline mutations in the *TP53* gene results in Li-Fraumeni syndrome, a rare genetic disorder characterized by the development of multiple tumours early in life.[176]

1.4.2 Mutational Signatures and Genomic Subtypes of Pancreatic Cancer

In recent years, advances in NGS technologies have allowed investigators to detect thousands of somatic mutations in a single cancer sample, revealing unprecedented insight into the diversity and complexity of the somatic mutational processes that underlie cancer development and progression. These mutational processes leave a characteristic pattern, or "mutational signature", on the cancer genome, which is defined by the type of DNA damage that was incurred, as well as the DNA repair and replication processes that were active (or inactive).[289]

In 2013, Alexandrov and colleagues evaluated the mutational spectrums of over 7000 primary cancers of 30 different cancer types, and defined 21 mutational signatures (signatures 1-21) that validated across tumour types based on the pattern and prevalence of 96 base substitution classes, defined by the six types of base substitutions (C>A, C>G, C>T, T>A, T>G, T>G) and sequence context immediately 5' and 3' to each mutated base.[295] In this study, 4 mutational signatures were identified in PAC, including signatures 1B, 2, 3 and 6.[295] Of special note is signature 3, which exhibits an approximately equal representation of all 96 substitution types, as

well as prominent numbers of large (up to 50 base pair) deletions with overlapping microhomology at deletion breakpoints.[295] Signature 3 was identified in breast, ovarian and pancreas cancer and was strongly associated with *BRCA1* and *BRCA2* mutations.[295] In fact, nearly all cases with *BRCA1* and *BRCA2* mutations showed a strong contribution from signature 3.[295] There were, however, cases with prominent signature 3 that did not harbour *BRCA1* or *BRCA2* mutations.[295]

In 2015, Waddell and colleagues examined patterns of structural variation (chromosomal rearrangement) in 100 PACs using WGS and copy number variation (CNV) analysis and defined 4 genomic subtypes of PAC based on the distribution and frequency of these rearrangements.[37] The "stable" genomic subtype accounted for 20% of cases, was characterized by 50 or fewer structural variation events and often demonstrated widespread aneuploidy, pointing to possible defects in the cell cycle or mitosis.[37] The "locally rearranged" subtype, accounting for 30% of cases, exhibited a significant focal event on one or two chromosomes.[37] About a third of these "locally rearranged" genomes contained copy number gains in regions that included known oncogenes.[37] The remaining genomes in this subtype contained complex local rearrangements such as breakage-fusion-bridge or chromothripsis events.[37] The "scattered" subtype was the most common of the 4 subtypes, accounting for 36% of all samples, and was characterized by a moderate number of non-random chromosomal damage and fewer than 200 structural variation events.[37] Finally, the "unstable" subtype, which accounted for 14% of samples, demonstrated vast genomic instability that was defined by more than 200 structural variation events, pointing to likely defects in DDR or DNA repair.[37] Fittingly, all 11 tumours in the case series with mutations in *BRCA1*, *BRCA2* or *PALB2* (either germline or somatic) fell within either the unstable genomic subtype or within the *BRCA* mutational signature previously described by Alexandrov and colleagues (signature 3),[295] with the majority (9 of 11) exhibiting both genomic features.[37] Notably, whereas

all *BRCA1* and *BRCA2* mutations exhibited bi-allelic inactivation, neither of the two germline *PALB2* mutation carriers had evidence of a somatic second hit.[37] Another interesting finding was that only about half of the tumours with unstable genomes and/or high *BRCA* mutational signature were accounted for by mutations in *BRCA1*, *BRCA2* or *PALB2*, suggesting that there may be other genes contributing to this genomic phenotype.[37] Silencing of these three genes by hypermethylation was ruled out as a contributing mechanism.[37] Mutations in other genes involved in DNA maintenance were observed including *RPA1*, *REV3L*, *ATM*, *FANCM*, *XRCC4* and *XRCC6*.[37]

In 2016, Connor and colleagues used WGS to evaluate the mutational signatures, as defined by Alexandrov and colleagues,[295] of 154 PAC cases.[296] Using hierarchical clustering according to the proportion of single-nucleotide variants (SNVs) attributable to each signature, the group defined 4 major PAC subtypes: "age-related", "double-strand break repair (DSBR)", "mismatch repair (MMR)" and "signature 8".[296] A replication cohort of 95 additional PAC cases independently identified the same 4 subtypes.[296] The DSBR subtype was characterized by signature 3 and cases within this subtype had increased numbers of both large structural and short deletions (3-20 bp) – genomic aberrations that are consistent with dysfunctional HR.[296] Combining the data from the discovery and replication cohorts in this study, 27 cases (10.8%) were of the DSBR subtype, of which 15 (56%) were explained by biallelic inactivation of *BRCA1*, *BRCA2* or *PALB2*, including 11 with pathogenic germline mutations and somatic inactivation of the wild-type allele, and 4 with somatic biallelic silencing.[296] Similar to the findings of Waddell and colleagues,[37] the remaining DSBR cases (approximately half) could not be explained by inactivation of *BRCA1*, *BRCA2* or *PALB2*.[296] Notably, two cases with germline *BRCA2* pathogenic mutations that lacked a somatic second hit, did not exhibit signature 3.[296] Another interesting

finding from this study was that cases within the DSBR, and especially MMR, subtypes had high levels of tumour-specific neoantigens that corresponded with elevated local antitumour immune activity by transcriptional and immunohistochemical analyses, a feature that might make these tumours amenable to immunotherapy.[296]

In addition to these genomic and mutational subtypes of PAC, whole transcriptome sequencing, which assesses gene expression, has resulted in the classification of several transcriptomic subtypes of PAC as described by Collisson and colleagues,[297] Moffitt and colleagues[298] and Bailey and colleagues[299]. While these transcriptomic subtypes have been associated with prognostic significance, they appear not to bear any relation to the genomic subtypes previously described, nor have any therapeutic implications been described, and these will not be discussed further in the present dissertation.

1.4.3 Precision Oncology Strategies in DNA Repair Deficient Pancreatic Cancer

The recent NGS efforts in PAC that were described in the section 1.4.2 have revealed a previously underappreciated intertumoural heterogeneity in PAC that cannot be clinically or histologically discerned. This might in part explain why previous clinical trials of novel therapies or novel combinations of therapies in PAC have been largely negative, reflecting perhaps poor patient selection and not poor drug efficacy.

These studies have also defined a distinct subtype of PAC characterized by genomic instability (signature 3/“unstable”/“DSBR”), often attributable to germline mutations in *BRCA1*, *BRCA2* or *PALB2*, which may be amenable to precision oncology approaches that exploit this vulnerability, such as DNA cross-linking agents and a newer class of enzyme inhibitors called poly(ADP-ribose) polymerase (PARP) inhibitors.

Among the DNA cross-linking agents are platinum salts, which include cisplatin, carboplatin or oxaliplatin, and mitomycin C, which act by inducing covalent bonds within the DNA double helix that stall the progression of the replication fork, ultimately leading to collapse of the fork and the formation of DSBs.[300] In cells with proficient HR, these DSBs are repaired without error, allowing the cell to survive. In cells with ineffective HR, such as in BRCA1-, BRCA2- or PALB2-deficient tumours, the cells must rely on the more error-prone single-strand annealing or NHEJ to repair the DSBs, which exacerbates genomic instability beyond cell viability.[301]

In breast and ovarian cancer, numerous preclinical and clinical studies have demonstrated that patients with *BRCA1* and *BRCA2* mutations have increased sensitivity to DNA cross-linking agents.[302-309] A similar role for targeted therapy with DNA cross-linking agents in *BRCA1*-, *BRCA2* or *PALB2*-deficient PAC is supported primarily by case reports,[37,310-312] *in vitro* studies,[313,314] preclinical *in vivo* studies in murine models,[37,314-316] and retrospective clinical data.[317,318]

Interestingly, in the study by Waddell and colleagues who described the "unstable" genomic subtype of PAC, 4 of 5 patients with unstable genomes and/or high *BRCA* mutational signature burden that were treated with platinum-containing therapy had measurable responses (2 had complete radiological responses and 2 had partial responses based on RECIST1.1 criteria), and 3 patients who did not exhibit either of these genomic features did not respond to platinum therapies.[37] Consistently, 2 of 3 patient-derived xenografts (PDXs) with these genomic features demonstrated sensitivity to cisplatin, while 4 PDXs without these features did not.[37] Taken together, tumours with unstable genomes and/or high *BRCA* mutational signature burden were significantly associated with response to platinum-based therapy (P=0.007, Fisher's exact test).[37]

A retrospective case series study by Golan and colleagues, who reported the largest cohort of *BRCA1* and *BRCA2*-associated PAC to date, found that among patients with late stage (III/IV) PAC and germline *BRCA1* or *BRCA2* mutations (n=43), those treated non-experimentally with a platinum-containing regimen (mostly gemcitabine in combination with cisplatin) had improved overall survival compared to those who did not receive a platinum (22 *versus* 9 months, *P*=0.039).[317] A more recent study by the same group, this time evaluating patients with resected (stage I/II) PAC and comparing patients with and without a *BRCA1* or *BRCA2* germline mutation, found no difference in overall survival between carriers and non-carriers.[318] They did however observe a trend towards increased disease-free survival among carriers compared to non-carriers treated with platinum-containing regimens (39.1 *versus* 12.4 months, *P*=0.255), where sample size may have been limiting.[318]

Whereas platinum salts directly overwhelm cells with DSBs, PARP inhibitors act in a synthetic lethal fashion by inactivating a second DNA repair pathway, which only when combined with ineffective HR, is lethal to the cell.[301] PARP inhibitors, such as olaparib and valiparib, are small molecule inhibitors of either PARP1 or PARP2, proteins that are essential for BER and the repair of SSBs that arise spontaneously during normal cellular activity. This inhibition results in persistent SSBs that become converted to DSBs during cell replication.[301] As a result, HR-deficient cells become overwhelmed with DNA damage that ultimately triggers cell death. Since non-cancerous cells retain one functional copy of *BRCA1*/*BRCA2*/*PALB2* and therefore the ability to repair DSBs by HR, PARP inhibitors act to selectively kill cancer cells with dysfunctional HR.[319]

PARP inhibitors have been shown to be efficacious in clinical studies of *BRCA1*- and *BRCA2*-associated breast and ovarian cancer.[320-326] In 2015, olaparib monotherapy received FDA approval for use in patients with advanced ovarian cancer and a germline *BRCA1* or *BRCA2*

mutation who have been treated with more than three prior lines of chemotherapy.[325] In *BRCA1*-, *BRCA2*- or *PALB2*-deficient PAC, sensitivity to PARP inhibitors have been demonstrated in a preclinical study by our group,[314] case reports,[327] as well as in early (phase I and II) clinical trials.[326,328,329] Several phase II and III clinical trials evaluating the efficacy of PARP inhibitors, alone or in combinations with a platinum, in *BRCA1*-, *BRCA2*- or *PALB2*-deficient PAC are currently ongoing.

Despite the role of ATM in the activation of HR, there is less evidence to support a role for DNA cross-linking agents and PARP inhibitors in ATM-deficient PAC. The genomes of ATM-deficient PAC tumors rarely exhibit the genomic hallmarks associated with HDR-deficiency.[37,296] In the study by Connor and colleagues, only 1 of 16 cases with bi-allelic *ATM* inactivation exhibited the genomic signature associated with HDR-deficiency.[296] While ATM-deficient breast cancer, prostate cancer and mantle cell lymphoma have been shown to have sensitivity to olaparib,[330-332] the chemotherapeutic sensitivities to platinum-based agents and PARPis in ATM-deficient PAC have yet to be investigated in either preclinical or clinical studies.

As research efforts continue to elucidate the genetic and genomic landscape of PAC, additional opportunities for precision oncology strategies may emerge.

1.5 Pancreatic Cancer Research Registries

Both the rarity and the rapidly fatal course of PAC pose major challenges for investigators aiming to study the etiology of PAC, hindering the collection of biospecimens and high-quality pedigree, clinical and epidemiologic data. The challenge is greater in studies of FPC, where the acquisition of samples from multiple PAC-affected family members is seldom achieved. Prospective familial research registries attempt to overcome these challenges, allowing for the collection of rare high-risk PAC families over time, with accompanying pedigree data and biospecimens. Registries with ongoing follow-up have the added potential of identifying and acquiring samples from new incident cases of PAC in family members who were unaffected at the time of initial enrolment. These prospectively followed high-risk cohorts are valuable in the study of disease penetrance, as well as in studies of early-detection biomarker and imaging-based screening modalities.

Indeed, much of our current understanding of FPC was made possible because of FPC research registries. Perhaps the largest FPC registry, the National Familial Pancreas Tumour Registry which was established in 1994 at Johns Hopkins Hospital in the United States,[82] has grown to include over 4,400 families, including over 1,400 FPC families, and has made substantial contributions to the field, including genetic risk quantification, as well as the discovery of novel PAC susceptibility genes.[66,82,136,137,236] Numerous other PAC registries have been established in North America and Europe.[241,333-336] Collaborations across family registries, such as the Pancreatic Cancer Genetic Epidemiology (PACGENE) Consortium,[337] which includes 8 North American PAC research registries, are likely to provide even greater power for gene discovery studies.

Another benefit of research registries in PAC is that it allows for the collection and characterization of rare subtypes of PAC, for example, PAC associated with germline *BRCA1* and *BRCA2* mutations, as well as other rare hereditary syndromes.

Familial registries vary in their recruitment strategies and inclusion criteria. Recruitment may be population-based, clinic-based, referral-based, or a combination of these methods. Data and sample collection typically includes a detailed family history and a blood or saliva sample from which surrogate germline DNA is extracted, from PAC-affected probands, as well as from affected and unaffected family members. Some registries expand data and sample collection to include archived and/or fresh pathology specimens, clinical and epidemiologic data.

Because of the rapid demise of patients with PAC, population-based PAC registries, which typically involve contacting patients by mail, have reported participation rates of 35% to 56%, with a significant proportion of PAC patients being deceased at the time of attempted contact (28% to 44%)[226,333,338]. In contrast, the Mayo Clinic's clinic-based Pancreatic Cancer Genetic Study, whose "ultra-rapid" recruitment strategy enrolls participants at the time of their first clinical encounter, has reported a much higher participation rate of over 75%.[334]

Prior to 2012, only a single PAC research registry existed in Canada – the Ontario Pancreas Cancer Study (OPCS), a population-based registry that was established in 2003.[333] Given the outstanding genetic questions in the field and the unique genetic demography of Quebec, the establishment of a PAC research registry in Quebec would provide a valuable research resource for studies of PAC heredity.

1.6 Rationale, Hypothesis and Aims

PAC is a lethal malignancy with only marginal improvement in survival outcomes in the last several decades.[11,339,340] Only a fraction of PAC cases (~20%) present with early stage disease that is amenable to surgical resection, which is currently the only chance for cure.[341] The median survival for patients presenting with metastatic disease is in the range of months.[342] The failure of current therapies and past clinical trials for novel therapies might reflect disease heterogeneity that is yet to be fully characterized. Given these dismal statistics, there is an urgent need for advances in early detection and precision oncology strategies for PAC.

Approximately 10% of PAC cases are attributable to Mendelian inheritance.[343] Only a small fraction of these cases are explained by known cancer syndromes, and a major question in the field remains the identification of novel PAC predisposition genes.[223,224] Among the known PAC predisposition genes are several genes important in DNA repair, including *BRCA1*, *BRCA2*, *PALB2* and *ATM*.[223,224] While germline mutations in *BRCA1, BRCA2* and *PALB2* are rare causes of PAC, they may have a more important role in populations enriched with founder mutations, like the French-Canadian (FC) population of Quebec, where recurrent "founder" mutations in these genes have been described.[195-198,200,201,344] While these founder mutations have been well characterized in patients with hereditary breast and ovarian cancer syndrome in the FC population,[195-198,200,201,344] the prevalence of these mutations among PAC cases in Quebec has not been investigated and there are no provincial guidelines for genetic testing for hereditary PAC risk.

As sequencing technologies have advanced in the last decade, characterization of genetic/genomic subtypes of cancers, including PAC, represent an exciting area of research, with promise for precision oncology strategies. In fact, PAC associated with germline mutations in *BRCA1*, *BRCA2* and *PALB2*, have been shown to exhibit a characteristic "unstable" genomic

signature that is reflective of their DNA double-stranded break repair (DSBR) deficiency,[37,296] and are sensitive to targeted therapies that exploit this DSBR deficiency, such as platinums and poly(ADPribose) polymerase inhibitors.[37,303,311,314]

A major focus of our lab is to elucidate the full spectrum of genetic causes of PAC, with the goal of improving clinical outcomes for PAC through improved risk stratification for early detection screening programs and characterization of PAC subtypes for precision oncology strategies. A hindrance of such genetic studies of PAC, however, is a lack of biospecimens, as well as high-quality family history, epidemiologic and clinical data, due to the rarity and rapid lethality of the disease. To address these research resource needs, our lab established the Quebec Pancreas Cancer Study, a prospective, clinic-based research registry and accompanying biospecimen repository, in April 2012.

The overarching hypothesis of my PhD dissertation is that ***a prospective, clinic-based PAC research registry can be used as a model to characterize the hereditary causes of PAC and identify strategies for early detection and precision oncology.*** The specific aims of my dissertation were to:

1) demonstrate that a clinic-based PAC research registry can be established with high participation rates and validate its utility as a resource to study the hereditary causes of PAC in Quebec (Chapter 2),
2) characterize the contribution of germline *BRCA1*, *BRCA2*, *PALB2* and *ATM* mutations to incident PAC in founder and non-founder populations (Chapter 3),
3) and identify novel PAC susceptibility genes in high-risk PAC cases using an exome sequencing approach (Chapter 4)

Chapter 2: Establishing a clinic-based pancreatic cancer and periampullary tumour research registry in Quebec

2.1 PREFACE TO CHAPTER 2

The combined rarity and rapid lethality of PAC has resulted in a paucity of biospecimens, as well as high-quality clinical and epidemiologic data, posing a major challenge for etiologic studies of PAC. These challenges are even greater for studies of PAC heredity, as familial and hereditary PAC represent only ~10-15% of cases,[65,223,224] and the collection of samples from multiple PAC-affected family members is difficult to obtain.[82] These challenges highlight the need for prospective PAC research registries and biobanks for studies of PAC heredity.

In the last two decades, several such PAC research registries have been established in North America and Europe,[241,333-336] and while these registries have resulted in substantial research contributions,[82] including the identification of novel PAC susceptibility genes,[136,137] there remain many outstanding questions in the field of PAC heredity.

When I began my graduate studies in 2012, the establishment of the Quebec Pancreas Cancer Study (QPCS), a prospective, clinic-based PAC research registry, was the research focus of our lab, which I took on as the first research aim of my thesis. This represented the first and only PAC research registry in Quebec and only the second in Canada, after the Ontario Pancreas Cancer Study (OPCS).[333] Importantly, the QPCS was designed to collect similar epidemiological and clinical variables as the OPCS, with the goal of fostering research synergy and collaboration across these two Canadian PAC research programs.

A unique contribution of a PAC research registry in Quebec is the inclusion of patients with French-Canadian ancestry, a population known to harbour recurrent "founder" mutations in several known disease-causing genes, including PAC susceptibility genes – *BRCA1*, *BRCA2* and *PALB2*,[195,196,199] and perhaps novel PAC susceptibility genes that have yet to be discovered.

Since the rapid progression of PAC is a major limiting factor in enrolment of patients in research studies, the QPCS was designed as a prospective, clinic-based study, as we hypothesized that this would lead to high participation rates and assimilation of high-quality clinical and epidemiologic data, and biospecimens. While the focus of the QPCS is PAC, the registry was expanded to include related peri-ampullary tumours (PATs), which are also underrepresented in research and difficult to treat.

Chapter 2 describes the rapid ascertainment methodology used by the QPCS, summarizes the results obtained following the first 374 referrals, and highlights the potential of the QPCS as a dynamic research resource for both independent and collaborative initiatives to further elucidate the genetics of PAC and associated translational impacts.

2.2 ABSTRACT

Background

Enrolling patients in studies of pancreatic ductal adenocarcinoma (PDAC) is challenging because of the high fatality of the disease. We hypothesized that a prospective clinic-based study with rapid ascertainment would result in high participation rates. Using that strategy, we established the Quebec Pancreas Cancer Study (QPCS) to investigate the genetics and causes of PDAC and other periampullary tumours (PATs) that are also rare and underrepresented in research studies.

Methods

Patients diagnosed with PDAC or PAT were introduced to the study at their initial clinical encounter, with a strategy to enrol participants within 2 weeks of diagnosis. Patient self-referrals

and referrals of unaffected individuals with an increased risk of PDAC were also accepted. Family histories, epidemiologic and clinical data, and biospecimens were collected. Additional relatives were enrolled in families at increased genetic risk.

Results

The first 346 completed referrals led to 306 probands being enrolled, including 190 probands affected with PDAC, who represent the population focus of the QPCS. Participation rates were 88.4% for all referrals and 89.2% for PDAC referrals. Family history, epidemiologic and clinical data, and biospecimens were ascertained from 91.9%, 54.6% and 97.5% respectively of patients with PDAC. Although demographics and trends in risk factors in our patients were consistent with published statistics for PDAC, the QPCS is enriched for families with French-Canadian ancestry (37.4%), a population with recurrent germline mutations in hereditary diseases.

Conclusions

Using rapid ascertainment, a PDAC and PAT research registry with high participation rates can be established. The QPCS is a valuable research resource and its enrichment with patients of French-Canadian ancestry provides a unique opportunity for studies of heredity in these diseases.

2.3 INTRODUCTION

Pancreatic ductal adenocarcinoma (PDAC) is a lethal malignancy with a 5-year survival rate of only 6%.[339,342,345] It represents the 4th leading cause of cancer-related death in Quebec, with an estimated 1,290 new cases diagnosed and 1,170 deaths in 2014.[345] These dismal statistics are attributable largely to late diagnosis: 80% of patients present with locally advanced or metastatic

disease that precludes curative-intent surgical resection.[341] The therapeutic options currently available for such patients are largely ineffective, and even patients who present with operable disease have poor outcomes because of early distant and local recurrences.[346]

Despite those tragic statistics, PDAC has, compared with other major cancers, been underrepresented in research studies largely because of its rapid progression and fatality – median survival being less than 4 months in the presence of metastatic disease.[342] As a result, high-quality epidemiologic data and biospecimens for PDAC research studies have been lacking. To overcome those challenges, several prospective PDAC research registries have been established in Europe and North America.[82] A resource of that calibre had not been established in Quebec, and the Ontario Pancreas Cancer Study (OPCS) was the only reported Canadian PDAC research registry.[333] Given the predominantly French-Canadian population in Quebec, a Quebec research registry would provide a unique resource for studies of heredity in PDAC, because founder genetic effects have been reported for a number of hereditary diseases in the French-Canadian population.[180]

To meet this research resource need in Quebec, we established a prospective clinic-based research registry, the Quebec Pancreas Cancer Study (QPCS). We used a clinic-based approach with a rapid ascertainment strategy because we hypothesized that those methods would facilitate high participation rates. The clinical and epidemiologic variables collected by the QPCS and OPCS overlap, with the goal of fostering research synergies between those two Canadian studies.

Although the primary objective of the QPCS is to establish a Quebec research resource for genetic susceptibility studies of PDAC, the data and biospecimens collected will also allow for future epidemiologic, biomarker, and cancer biology studies of PDAC. In addition, the QPCS includes cases of other periampullary tumours (PATs) that are also rare, underrepresented in

research studies, and often difficult to treat. Characterization of such cases could prove helpful in advancing our understanding of PDAC and in contributing to more global studies of biliary and gastrointestinal precancerous and cancerous conditions.[347,348]

Here, we describe the methods used to establish the QPCS and the results obtained after the first 374 referrals, with an emphasis on PDAC.

2.4 METHODS

2.4.1 Study Participants

2.4.1.1 Inclusion Criteria

Men and women more than 18 years of age with a diagnosis of PDAC or PAT were eligible to participate. Patients with disease confirmed by histology or cytology were included, as were patients diagnosed based on clinical and axial imaging when a biopsy was either unavailable or pending. Unaffected individuals with a family history of PDAC or a diagnosis of a PDAC-associated genetic syndrome (hereditary breast and ovarian cancer syndrome, hereditary pancreatitis, hereditary non-polyposis colorectal cancer, Peutz-Jeghers syndrome, or familial atypical multiple-mole melanoma)[346] were also eligible to participate.

Although PDAC is the primary focus of the QPCS, the registry was expanded in October 2012 to include PATs, including pancreatic neuroendocrine tumours, solid pseudo-papillary epithelial neoplasms, premalignant lesions of the pancreas (for example, intraductal papillary mucinous neoplasm, mucinous cystic neoplasms), and pre-invasive and malignant biliary, ampullary, gastric and duodenal lesions. We defined “proband” as the first individual in a family to enrol in the QPCS, whether that individual was affected or unaffected. Institutional Research Board approval was obtained for the study.

2.4.1.2 Referrals and Enrollment

Patients with a diagnosis of PDAC or PAT were informed of the study by the treating hepatopancreatobiliary surgeon or oncologist at the time of their initial consultation at the McGill University Health Centre (MUHC). Patients interested in participating met with the QPCS study coordinator after their initial clinic visit or were contacted within 2 weeks for an in-person or telephone interview. Referrals from cancer genetics or oncology clinics outside of the MUHC, patient self-referrals, and referrals of unaffected individuals with a family history or known genetic syndrome associated with the earlier-described conditions were also accepted. Self-referrals occurred primarily through the QPCS Web page (http://www.cancerpancreas.ca). If, after 2 attempts (made twice monthly), a study candidate could not be reached in person or by telephone, and no response ensued during the following 6 months, the participant was classified as a "non-responder".

Probands (or their next-of-kin) are contacted annually after enrolment to obtain updated demographics, vital status, medical history, and genetic testing, as well as any changes in family history. Written informed consent from all participants was obtained at the time of enrolment.

2.4.1.3 Work Up of Families

With consent from the proband, relatives (both affected and unaffected at-risk blood relatives) were invited to enrol in the QPCS when the family history was consistent with an increased risk of PDAC or related conditions. If an affected family member was deceased, consent for the individual was obtained from the appropriate next-of-kin.

Genetic counselling and familial risk assessment were provided to each proband and family

after enrolment. Families with a history suggestive of a genetic syndrome were referred to clinical genetics.

2.4.2 Data and Biospecimen Collection

2.4.2.1 Family History

At the time of enrolment, study participants were asked to provide a detailed family history either by interview with a genetic counsellor or by completing a family history questionnaire. In either case, a three-generation pedigree capturing both the maternal and paternal branches of the family, with details about ethnicity and family history, was obtained.

2.4.2.2 Epidemiology

Study participants were asked to complete a personal history questionnaire (PHQ), which included detailed questions about lifestyle and epidemiologic risk factors. Data collection included medical history, medication use, dietary patterns, physical activity, reproductive history, chemical exposures, and alcohol and tobacco consumption. If a participant was deceased at the time of enrolment, the next-of-kin was asked to complete the PHQ on the participant's behalf.

2.4.2.3 Clinical Data

Participants provided the QPCS with written consent to obtain medical records for confirmation of their diagnosis (by radiological imaging or pathology report, or both) and for collection of data relating to clinical treatments and outcomes. For participants who presented with a prior diagnosis of a genetic syndrome, clinical genetics records were obtained to confirm mutation status. For deceased family members who had a diagnosis of cancer, consent to obtain medical records was obtained from the appropriate next-of-kin. We also obtained permission to access records collected by the Province of Quebec (Régie de l'assurance maladie du Québec),

which allows the QPCS to perform a death clearance analysis.

2.4.2.4 Biospecimens

At enrolment, probands and relatives were asked to provide blood (blood tubes: BD Biosciences, Mississauga, ON) or saliva samples (saliva collection kit: DNA Genotek, Ottawa, ON), or both, for biobanking. Blood samples were obtained either at a regular clinic appointment, by a research nurse at the time of enrolment, or preoperatively from participants undergoing surgical resection at the MUHC. Otherwise, (for example, if a participant resided out of town), a blood kit was mailed to the participant so that blood could be drawn at a clinic closer to the participant's home. Blood samples were then shipped and received within 24-48 hours. Alternatively, participants had the option to provide a saliva specimen, which was returned to the study coordinator at the time of enrollment or by mail. If a deceased participant had received clinical genetic testing, archived DNA samples from the testing laboratory were requested for biobanking.

Blood samples were processed within 3 hours (local blood draws) or within 24-48 hours (shipped samples) from the time of collection. Plasma samples (collected only in the case of local draws) were immediately placed on ice and processed within 3 hours of collection. White blood cells were separated using an ammonium chloride red blood cell lysis buffer, and lymphocytes were isolated using a gradient lymphocyte separation medium (Ficoll-Paque: GE Healthcare, Baie d'Urfe, QC; lymphocyte separation medium: Wisent Bioprodcuts, St-Bruno, QC). The plasma, white blood cell pellets and lymphocyte pellets were stored in liquid nitrogen. Extracted DNA was resuspended in Tris-EDTA buffer and stored at 4°C.

For participants with a diagnosis of PDAC or a related PAT, we aimed to obtain samples of the corresponding tissues for biobanking. With patient consent, we requested archived

(formalin-fixed, paraffin-embedded) tissue samples from biopsies or resection specimens (or both) from the treating hospitals. We also obtained any available archived non-tumour tissue samples that could be used as surrogate germline tissues for deceased participants enrolled by a next-of-kin.

Consent was obtained from patients undergoing resection at the MUHC for collection of fresh-frozen tissue samples for tissue biobanking. Samples were collected immediately after resection. A pathologist first examined each specimen macroscopically, confirmed the diagnosis, and determined if the tumour tissue was sufficient for biobanking without compromising the clinical pathology diagnosis. When possible, paired affected (that is, tumour) and adjacent unaffected (that is, "normal") tissues were obtained. Depending on availability and diagnosis, tumour samples collected from resection specimens were also used to establish patient-derived xenografts in immune-compromised mice.

2.4.2.5 Data Storage

Family history and pedigree data were stored and manipulated using a genetic data management system (Progeny Clinical, version 9: Progeny Software LLC, Delray Beach, FL, U.S.A.; http://www.progenygenetics.com/). All study participants were assigned a unique QPCS identification number to ensure anonymity and confidentiality. Biospecimen data were stored separately using the Canadian BioSample Repository (CBSR) software package (www.biosample.ca). Each biospecimen was assigned a database repository biobank number unique from the QPCS identification number. The QPCS repository is registered within the Canadian Tumour Repository Network.

2.5. RESULTS

Between April 1, 2012, and July 31, 2014, the QPCS received 374 patient referrals, of which 230 had PDAC (Table 2.1). Most referrals (88.5%) came from hepatopancreatobiliary surgery and oncology clinics; the remainder came from genetics clinics or were self-referrals. Because QPCS enrolment is ongoing, 28 referrals (7.5%) were pending enrolment at July 31, 2014. Of the completed patient referrals (that is, excluding the pending enrolments), 306 probands (88.4%) had been enrolled, 20 patients (5.8%) were nonresponders, 10 patients (2.9%) had declined to participate, and 10 patients (2.9%) were palliative or deceased at the time of attempted contact, translating into participation rates of 88.4% and 89.2% for all referrals and PDAC referrals respectively.

Among the 306 probands enrolled, 277 provided family history data (90.5%), and 172 (56.2%) completed the PHQ. In addition to probands enrolled, 56 relatives of probands from 25 families were also enrolled. Considering all subjects enrolled [n = 362 (that is, 306 probands and 56 relatives of probands)], the QPCS now has 198 participants with a PDAC diagnosis, 182 (91.9%) of whom have provided family history data, and 108 of whom (54.5%) gave also made epidemiologic and clinical data available. Notably, most PDAC-affected probands (69.5%) were enrolled within 3 months of their diagnosis.

Table 2.2 summarizes the diagnoses of enrolled probands. Of the 306 probands successfully enrolled, 190 (62.1%) had been diagnosed with PDAC; 36 (11.8%) with a related periampullary malignancy (distal or hilar cholangiocarcinoma; ampullary, gallbladder or duodenal cancer); 3 (1.0%) with gastric cancer; 27 (8.8%) with a premalignant pancreatic lesion (intraductal papillary mucinous neoplasm, mucinous cystic neoplasm); 3 (1.0%) with pseudo-papillary epithelial neoplasms; 11 (3.6%) with pancreatic neuroendocrine tumours; and 10 (3.3%) with

benign pancreatic lesions (for example, pancreatitis, microcystic serous adenoma). In one case, the final pathology diagnosis of the resected specimen was metastatic low-grade sarcoma (epithelioid hemangioendothelioma).

Of the 190 probands with a PDAC diagnosis, 52 (27.4%) had tumours that were resectable, 58 (30.5%) had locally advanced disease, and 80 (42.1%) had metastatic disease at the time of enrolment. The patients with benign surgical pathologies were enrolled based on premalignant or malignant preoperative clinical diagnoses. The preoperative diagnosis for the case with the low-grade sarcoma was PDAC. Multiple synchronous primary tumours were diagnosed in 2 probands. Additionally, 23 probands (7.5%) were unaffected, but were enrolled because of an increased risk of PDAC: that is, because of a significant family history, a mutation carrier with a known genetic syndrome (for example, Peutz-Jeghers), or in one case, a chronically elevated serum level (>500 U/ml) of the CA19-9 tumour biomarker that was found incidentally without radiologic evidence of a lesion.

The study has ascertained 668 tissue samples from 333 of the 362 total enrolled subjects. Whole blood (plasma and buffy layer) was collected from 237 subjects (71.2%) and buffy layer alone from 20 subjects (6.0%). A blood sample could not be collected from 69 subjects (20.7%); however, unaffected tissue samples in the form of saliva, DNA or non-tumour tissue specimens (formalin-fixed, paraffin-embedded or fresh-frozen) were obtained. Affected tissue biospecimens (formalin-fixed, paraffin-embedded or fresh-frozen, or both) were collected from 189 subjects (56.8%). Notably, both affected and unaffected tissue specimens were collected from 182 subjects (54.7%).

Considering only the 198 subjects with a diagnosis of PDAC, the study obtained 401 samples from 193 subjects (97.5%), including unaffected tissue samples from 188 subjects

(95.0%) and PDAC-affected tissue samples from 118 subjects (59.6%). For 113 PDAC-affected subjects (57.1%), the QPCS ascertained both non-tumour (that is, surrogate germline) and PDAC biospecimens. Notably, biospecimens have been collected from 56 relatives in 25 families with at least 1 PDAC-affected family member enrolled (n = 193), including biospecimens from 2 or more PDAC-affected family members (including the proband) in 6 families.

Table 2.3 describes the characteristics of the 198 enrolled subjects with a PDAC diagnosis (that is, PDAC-affected probands and PDAC-affected relatives). Demographic and epidemiologic data were curated using available family history and PHQ data from the PDAC subjects or their next-of-kin. Mean age at diagnosis was 66.1 ± 10.5 years, and the ratio of men to women was 1.4:1. Most subjects with PDAC were white (79.2% maternal, 80.1% paternal), with an enrichment of cases (37.4%) having French-Canadian ancestry (at least 1 parental origin, Table 2.3). In addition, 56.8% of PDAC-affected subjects had an ancestry (that is, French-Canadian, Ashkenazi Jewish, Greek, German, Polish, or Latvian) known to harbour recurrent germline ("founder") mutations in the *BRCA1*, *BRCA2* and *PALB2* PDAC predisposing genes (Table 2.3).[129,195,196,199,216,219,349-351] Table 2.3 also shows data describing education, environmental exposures, weight loss, and history of Type II diabetes and pancreatitis for enrolled patients with PDAC.

Table 2.4 summarizes the distribution of enrolled families with a high probability of hereditary PDAC. In families with an available family history and at least 1 case of PDAC or otherwise at risk of PDAC (n = 211), tumours in 152 (72.0%) were classified as sporadic because the family had no history of PDAC and did not meet criteria for a genetic syndrome associated with hereditary PDAC. Another 59 patients (28.0%) had an increased likelihood of hereditary PDAC, either because of multiple PDAC diagnoses in the family [that is, familial PDAC (fPDAC),

18.5%], because of diagnosis of a genetic syndrome whose tumour spectrum includes PDAC (3.8%), or because of young age of onset (diagnosed with PDAC at 50 years of age or younger, 5.7%). Those observations are consistent with the distribution of PancPRO scores for all PDAC probands with an available pedigree (n = 175, Figure 2.1). PancPRO is a risk prediction tool (courtesy of BayesMendel Lab, Harvard University, Cambridge, MA, U.S.A.) designed to estimate the probability that an individual carries a deleterious mutation in a PDAC susceptibility gene.[352]

2.6. DISCUSSION

PDAC research has been hampered by a paucity of data and biospecimens. Perhaps the most significant challenge in conducting PDAC studies is recruitment of cases, because of rapid progression and death after diagnosis. The QPCS was designed as a clinic-based study with the goal of rapid case ascertainment after a PDAC or PAT diagnosis. Using that approach, we achieved participation rates of 88.4% for all referrals and 89.2% for referrals with a PDAC diagnosis. Moreover, just 3.8% of PDAC cases were palliative or deceased at the time of attempted contact. Previous population-based PDAC registries have reported much lower participation rates of 35-56%, with a significant proportion of PDAC patients being deceased at the time of contact (28-44%).[333,338] In contrast to such low participation rates, the Mayo Clinic's clinic-based Pancreatic Cancer Genetic Study has reported enrolment rates (approximately 80%) similar to those achieved by the QPCS.[334] A comparison of study results supports our hypothesis that a clinic-based recruitment approach with an emphasis on rapid ascertainment facilitates high participation rates in PDAC studies.

Although the QPCS achieved high participation rates, the study has limitations associated with a clinic-based approach. The low proportion of palliative or deceased PDAC cases at

attempted contact may reflect a selection bias because our patients were referred primarily from surgery and oncology clinics, likely favouring patients with a higher performance status. The QPCS is also affected by the pattern of referrals to the MUHC. In 2003, the Quebec Ministry of Health and Social Services created the Réseau universitaire intégré de santé, assigning a portion of the province's territory to each of the four provincial medical schools. McGill's territory includes Nunavik, the Cree Territory, Nord du Québec, Abitibi-Témiscamingue, Outaouais, western Montéregie, and western Montreal. As such, the MUHC's referrals come largely from these regions of Quebec. Despite those probable biases, the demographics of the QPCS case series are consistent with previous reports in North America,[339,345,353] suggesting that the QPCS is an accurate representation of the patient population with PDAC. The inclusion, in addition to PDAC, of related PATs is a unique research resource. Further, the QPCS results demonstrate that research registries can be successfully integrated into high-volume tertiary care surgery and oncology clinics. Moreover, the enrolment of cases through surgery and oncology clinics provides unique opportunities for studies of disease heredity in the prospectively collected cases, unselected for genetic susceptibility.

A number of lifestyle and environmental risk factors are associated with PDAC. Although our cohort is currently small for epidemiologic analyses, we observed trends in risk factors associated with PDAC that are consistent with those previously reported.[346,354] We collected level of education as a surrogate for socioeconomic status, because low socioeconomic status has been associated with higher PDAC mortality rates.[355] Of participants who returned the PHQ (n = 109), 44.0% reported obtaining a high school diploma or less, and 56.0% indicated having received at least some college or university education. Smoking is the most common risk factor associated with PDAC. Consistent with previous reports,[346] more than half the PHQ questionnaire responders

(51.4%) reported a history of cigarette smoking. Notably, 78.5% were exposed either directly or indirectly to cigarette smoke in their lifetime (Table 2.3). Compared with abstention and occasional drinking, heavy alcohol consumption (≥6 drinks/day) has been associated with an increased risk of PDAC.[29] Of our PHQ responders, 70.1% indicated consumption of more than 1 alcoholic beverage weekly (mean: 10.0 ± 17.9 alcoholic beverages consumed weekly).

Long-standing type 2 diabetes (>10 years) has also been shown to increase risk of PDAC by a factor of 1.51, and new-onset diabetes (<3 years) can be an early symptom of disease.[30,33] Furthermore, individuals with a history of chronic pancreatitis (>2 years before the PDAC diagnosis) have a risk of PDAC that is increased by a factor of 2.71, and new-onset pancreatitis could be a symptom of PDAC-associated ductal obstruction.[32] In our study, 30 participants (28.9%) reported a diagnosis of type 2 diabetes before their PDAC diagnosis, with a mean of 8.9 ± 7.9 years between that diagnosis and their PDAC diagnosis (range: 0-25 years). A diagnosis of pancreatitis was reported by 6 participants (5.8%) at a mean of 8.0 ± 10.0 years before their PDAC diagnosis (range: 0-26 years). Although our cohort is small, those observations are consistent with the results of a pooled case-control analysis showing that 6.2% of PDAC patients have a history of pancreatitis.[32] Younger patients (<65 years of age) with a history of pancreatitis could be at increased risk for genetic predisposition to PDAC, including hereditary pancreatitis.[356]

Several studies have shown that an increased body mass index (BMI) is associated with PDAC risk.[28] In the present study, 74.8% of responders with PDAC were overweight (BMI > 25) and 14.5% were obese (BMI > 35) 1 year before enrolment. Interestingly, the average BMI for participants diagnosed with type 2 diabetes was higher than the average BMI for participants without diabetes, both at the time of enrolment (26.3 ± 4.20 vs. 25.6 ± 4.46) and at 1 year before enrolment (30.9 ± 5.29 vs. 29.0 ± 5.61). Because of discrepancies in time of enrolment relative to

the time of PDAC diagnosis, the latter observations might be underestimated, given that weight loss is a common symptom of PDAC. To account for that possibility, we also determined the mean BMI at enrolment and 1 year before enrolment (24.0 ± 4.66 and 27.2 ± 4.31 respectively) only for responders with PDAC who were enrolled within 3 months of diagnosis (n = 64). Because the assessment of lifestyle and environmental exposures was retrospective and self-reported, the possibility of recall bias cannot be excluded.

Research registries for PDAC have made important contributions to investigations of the genetic causes of PDAC, including the discovery of novel PDAC susceptibility genes.[82,136,137] Estimates suggest that 10% of PDAC cases are attributable to Mendelian inheritance.[343] Although a small fraction of such hereditary cases are explained by germline mutations in known PDAC susceptibility genes (for example, *BRCA1*, *BRCA2, PALB2, CDKN2A*),[343] one of the most important questions in field remains the identification of the genetic causes of fPDAC in which known genes are not implicated. The QPCS was designed with the goal of collecting high-quality data and biospecimens for genetic studies of PDAC. Importantly, we aimed to collect epidemiologic and clinical variables similar to those collected by the OPCS,[333] which will allow for research collaboration by the two Canadian studies. In addition, the QPCS design has allowed us to contribute data and biospecimens to the multicentre Pancreatic Cancer Genetic Epidemiology Consortium,[337] as part of a larger collaborative effort to elucidate the genetics underlying fPDAC.

Through our integrated genetic counselling program, the QPCS has obtained detailed pedigrees and family history data from 90.5% of enrolled probands, including 174 probands with PDAC (91.6%). High-risk PDAC families have been extensively characterized, including acquisition of biospecimens from affected and unaffected blood relatives. Enrolled families are followed prospectively by annual contact to monitor for new diagnoses in the family. As has been

the experience of other PDAC registries, the QPCS expects that its prospective approach will likely identify new (incident) cases of PDAC in family members who were unaffected at the time of enrolment, whether their kindred had multiple or only 1 affected relative at the time of QPCS enrolment.[82] Moreover, the ascertainment of both unaffected (that is, surrogate germline) and affected tissues in 113 cases of PDAC (57.1% of enrolled cases) is particularly valuable for genetic predisposition studies.[357] Finally, the concomitant collection of lifestyle and environmental exposure data for the participants will allow for rationalization of non-genetic contributors to PDAC causation.

Notably, more than half the enrolled patients in the QPCS affected with PDAC (56.8%) had an ancestry (at least 1 parental origin) known to harbour founder *BRCA1* and *BRCA2* mutations.[129,195,196,199,216,219,349-351] The founder populations represent a genetically enriched subgroup ideal for gene discovery studies.[180,358-360] The enrichment of the QPCS with participants of French-Canadian descent is particularly valuable because fPDAC is prevalent in that population.[58] In addition, French-Canadian founder mutations in the *BRCA1*, *BRCA2* and *PALB2* genes have already been described,[195,196,199] providing the QPCS with a unique opportunity to study the prevalence of those founder mutations among French-Canadians participants with PDAC. *BRCA1*- and *BRCA2*-associated PDAC is of particular interest because those tumours can have beneficial treatment responses to DNA cross-linking agents (that is, platinum salts) and PARP inhibitors.[303,311,320,361-363]

The immediate research directions of the QPCS include characterizing the hereditary contribution of PDAC. In particular, as a collaborative effort with the OPCS, we are using next-generation sequencing to search for novel PDAC susceptibility genes in fPDAC kindreds. Moreover, we will take advantage of the French-Canadian patient population collected by the

QPCS to look for recurrent genetic mutations in known PDAC susceptibility genes, because those results could have important implications for clinical genetic screening in that population.

2.7. CONCLUSIONS

We have demonstrated that a rapid ascertainment protocol in a clinic-based PDAC and PAT research study can achieve high participation rates of 88.4% for all referrals and 89.2% for PDAC referrals. After the OPCS,[333] the QPCS is the second PDAC research registry to be reported in Canada, and it provides a valuable resource available to the scientific community at large for studies of PDAC and related PATs. The registry will also facilitate identification in Quebec of eligible participants for clinical screening protocols as new imaging technologies and biomarkers emerge.

2.8. ACKNOWLEDGEMENTS

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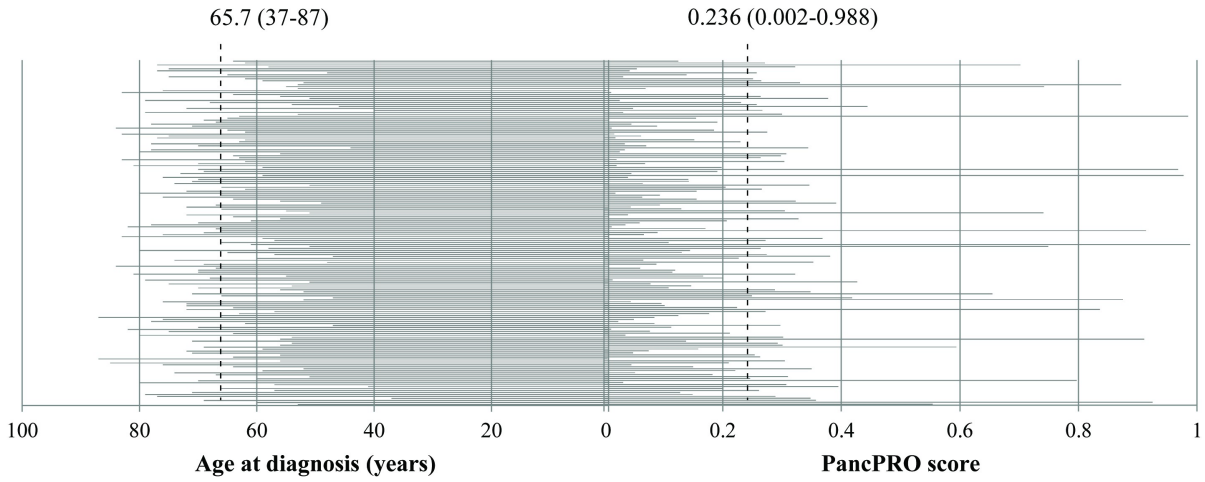
2.9 FIGURES

Figure 2.1. Ages at diagnosis (in years) and PancPRO scores for all enrolled probands with a diagnosis of pancreatic ductal adenocarcinoma and an available pedigree (n = 175). Dashed lines indicate the mean age and the mean PancPRO score (ranges given in parentheses). PancPRO model courtesy of BayesMendel Lab, Harvard University, Cambridge, MA, U.S.A.



2.10 TABLES

Table 2.1. Quebec Pancreas Cancer Study referrals and enrolment between April 1, 2012, and July 31, 2014.

Variable	*Patients [*n *(%)]*	
	With PDAC	*Overall*
Referrals	230	374
HPB surgery and oncology clinics	218 (94.8)	331 (88.5)
Genetics clinics	9 (3.9)	23 (6.2)
Self-referral	3 (1.3)	20 (5.4)
Pending enrolment	16 (7.0)	28 (7.5)
Completed referrals	213 (92.6)	346 (92.5)
Enrolled[a]	190 (89.2)	306 (88.4)
At diagnosis[b]	132 (69.5)	-
>3 months after diagnosis[b]	58 (30.5)	-
Family history[b]		
Complete	174 (91.6)	277 (90.5)
Pending	11 (5.8)	20 (6.5)
Unavailable	5 (2.6)	9 (2.9)
Personal history[b]		
Complete	107 (56.6)	172 (56.2)
Pending	30 (15.8)	48 (15.7)
Unavailable	53 (27.9)	86 (28.1)
Non-responders[a]	8 (3.8)	20 (5.8)
Declined[a]	7 (3.3)	10 (2.9)
Palliative/deceased[a]	8 (3.8)	10 (2.9)

[a] Percentages calculated on completed referrals.
[b] Percentages calculated on enrolled pateints.
PDAC = pancreatic ductal adenocarcinoma;
HPB = hepatoancreatobiliary.

Table 2.2. Diagnoses of enrolled probands (*n* = 306).

Diagnosis	*Probands [*n *(%)]*
Pancreatic ductal adenocarcinoma (PDAC)	190 (62.1)
Distal cholangiocarcinoma	12 (3.9)
Hilar cholangiocarcinoma	3 (1.0)
Ampullary cancer	12 (3.9)
Gallbladder cancer	6 (2.0)
Duodenal cancer	3 (1.0)
Gastric cancer	3 (1.0)
Multiple synchronous primaries	2 (0.6)
Distal cholangiocarcinoma and gallbladder cancer	1 (0.3)
Pancreatic neuroendocrine tumor and colorectal cancer	1 (0.3)
Epithelioid hemangioendothelioma metastatic to the pancreas	1 (0.3)
Mucinous cystic neoplasm of the pancreas	3 (1.0)
Intraductal papillary mucinous neoplasm of the pancreas	24 (7.8)
Side branch	15 (4.9)
Main branch	9 (2.9)
Pseudopapillary pancreatic tumor	3 (1.0)
Microcystic serous adenoma of the pancreas	5 (1.6)
Pancreatic neuroendocrine tumor	11 (3.6)
Pancreatitis	5 (1.6)
Unaffected, with increased risk of PDAC	23 (7.5)
Family history of pancreatic malignancy	16 (5.2)
Genetic syndrome diagnosis	6 (2.0)
Elevated CA19-9	1 (0.3)

Table 2.3. Demographics and epidemiology of 198 enrolled patients with pancreatic ductal adenocarcinoma (PDAC).

Characteristic	*Pts*[a] *(*n*)*	*Value*
Mean age (years)	198	66.1±10.5
Gender (*n* [%])	198	
Men		115 (58.1)
Women		83 (41.9)
Education status (*n* [%])	109	
High school or less		48 (44.0)
Some college/university		13 (11.9)
College or university graduate		23 (21.1)
Postgraduate degree		25 (22.9)
Ancestry with founder *BRCA1* and *BRCA2* mutations (*n* [%])	190	108 (56.8)
French-Canadian		71 (37.4)
Ashkenazi Jewish		21 (11.1)
Greek		8 (4.2)
German		5 (2.6)
Polish		2 (1.1)
Latvian		1 (0.5)
Weight change	103	
Loss during the year preceding enrolment (*n* [%])		89 (86.4)
Mean loss during the year preceding enrolment (kg)		11.8±23.5
Mean BMI 1 year before enrolment (kg/m^2)		29±5.61
Mean BMI at enrolment (kg/m^2)		25.6±4.5
BMI > 25 kg/m^2 1 year before enrolment (*n* [%])		77 (74.8)
BMI > 25 kg/m^2 at enrolment (*n* [%])		53 (51.5)
Patients with type 2 diabetes	104	
Diabetes diagnosed before PDAC (*n* [%])		30 (28.8)
Mean time of diagnosis before PDAC (years)		8.9±7.9
Mean BMI 1 year before enrolment (kg/m^2)		30.9±5.3
Mean BMI at enrolment (kg/m^2)		26.3±4.2
Patients with pancreatitis	103	
Pancreatitis diagnosed before PDAC (*n* [%])		6 (5.8)
Mean time of diagnosis before PDAC (years)		8±10.0
Environmental exposures	107	
Tobacco		
Nonsmoker (*n* [%])		52 (48.6)
Former smoker (*n* [%])		47 (43.9)
Current smoker (*n* [%])		8 (7.5)
Smoking history (mean years)		24.7±14.6
Mean cigarettes daily (*n*)		19.9±27.5
Exposed to second-hand smoke ≥1 hour daily for more than ≥10 years (*n* [%])		83 (77.6)
Alcohol		
Consumer of >1 alcoholic beverage weekly (*n* [%])		75 (70.1)
Duration of consumption of >1 beverage weekly (mean years)		32±15.4
Mean alcoholic beverages weekly		10±17.9

[a] For whom data are available

Pts = patients; BMI = body mass index

Table 2.4. Predisposition to pancreatic ductal adenocarcinoma (PDAC) in 211 enrolled families[a] with available family histories.

Classification	*Families [*n *(%)]*
Sporadic PDAC	152 (72.0)
Non-sporadic PDAC	59 (28.0)
Familial PDAC[b]	39 (18.5)
2 Affected relatives	34 (16.1)
3 Affected relatives	2 (0.9)
≥4 Affected relatives	3 (1.4)
Genetic syndrome diagnosis	8 (3.8)
Hereditary breast and ovarian cancer (*BRCA1*)	3 (1.4)
Hereditary breast and ovarian cancer (*BRCA2*)	2 (0.9)
Hereditary breast and ovarian cancer-like (*PALB2*)	1 (0.5)
Familial atypical multiple-mole melanoma (*CDKN2A*)	1 (0.5)
Peutz-Jeghers syndrome (*STK11*)	1 (0.5)
Young-onset PDAC (≤50 years)	12 (5.7)

[a] With 1 or more cases of PDAC (n = 209) or otherwise at risk of PDAC (n = 2).
[b] Defined as 2 or more PDAC-affected relatives in a family, without diagnosis of a known genetic syndrome.

Chapter 3: Reflex testing for germline *BRCA1*, *BRCA2*, *PALB2* and *ATM* mutations in pancreatic cancer: mutation prevalence and clinical outcomes from two Canadian research registries.

3.1 PREFACE TO CHAPTER 3

Chapter 2 described the establishment of the QPCS, a prospective, clinic-based PAC research registry with accompanying biospecimen repository, which is the first and only PAC registry in Quebec.[364] We demonstrated that the QPCS captures PAC cases at "high-risk" of hereditary PAC, and is enriched for cases with French-Canadian ancestry (37%).[364] We also described the synergism in data collection between the QPCS and the OPCS, and highlighted the potential for collaborative research studies.[364]

In Chapter 3, we used the QPCS and OPCS resources to investigate the translational significance of reflex genetic testing (that is, automatic testing of all incident cases at diagnosis) for germline mutations in 4 known PAC susceptibility genes associated with homology-directed DNA repair (HDR; *BRCA1*, *BRCA2*, *PALB2* and *ATM*) among 368 consecutive PAC cases. Our study was focused on these 4 genes since they are likely the most prevalent genes implicated in hereditary PAC, and since carriers of mutations in these genes may benefit from therapies that specifically target HDR deficiency. Such therapies include platinum salts (e.g., cisplatin) and PARP inhibitors, which have been shown to be efficacious in preclinical studies of *BRCA1*-, *BRCA2*- and *PALB2*-associated PAC,[37,313-316] including a study by our lab in 2015.[314] A previous retrospective clinical study has also suggested improved clinical outcomes for *BRCA1*- and *BRCA2*-associated PAC treated with platinums.[317] Given the rapid progression and chemoresistance of PAC, the timely identification of mutation carriers (i.e., soon after diagnosis) is a requisite step in guiding such precision oncology decisions, which may translate to improved clinical outcomes for this genetic subtype of PAC. [317,318]

While previous studies have evaluated the prevalence of mutations in these genes in incident PAC,[134,135] these studies have included smaller sample sizes or have been largely limited

to *BRCA1* and *BRCA2*, and have not reported on the clinical outcomes of carriers. An important question in the field remains whether reflex testing should be offered to all or select incident PAC cases.

Unique to our study is the inclusion of a large case series of patients with French-Canadian ancestry (n=132), a population known to harbour 20 founder mutations in *BRCA1*, *BRCA2* and *PALB2*.[195-198,200,201,344] While these mutations have been well-studied in the context of hereditary breast and ovarian cancer, the contribution of these mutations to PAC among French-Canadians had not been previously investigated.

In Chapter 3, we performed comprehensive genetic analyses to assess the prevalence of mutations in *BRCA1, BRCA2, PALB2* and *ATM*, and compared relevant family history and clinical variables, as well as overall survival, between carriers and non-carriers. Our findings consolidate the current literature in the field of PAC associated with germline mutations in HDR genes, define the prevalence of founder mutations in these genes among patients with French-Canadian ancestry, provide supporting evidence for a distinct clinical subtype of PAC with improved outcomes, and finally, propose simple screening criteria for reflex genetic testing that are practical to apply in an ambulatory clinic setting by surgeons and oncologists.

3.2 ABSTRACT

PURPOSE: We investigated the translational value of reflex testing for germline mutations in four homology-directed DNA repair predisposition genes (*BRCA1*, *BRCA2*, *PALB2* and *ATM*) in consecutive patients with pancreatic adenocarcinoma.

PATIENTS AND METHODS: One hundred fifty patients with French-Canadian (FC) ancestry were evaluated for founder mutations, and 114 patients were subsequently assessed by full gene

sequencing and multiplex ligation-dependent probe amplification for nonfounder mutations. Two hundred thirty-six patients unselected for ancestry were also assessed for mutations by full gene sequencing.

RESULTS: The FC founder mutation prevalence among the 150 patients was 5.3% (95% CI, 2.6% to 10.3%), and the nonfounder mutation prevalence across the four genes among the 114 patients tested was 2.6% (95% CI, 0.6% to 7.8%). In the case series unselected for ancestry, 10.0% (95% CI, 2.7% to 26.4%) of patients reporting Ashkenazi Jewish (AJ) ancestry carried an AJ founder mutation, with no nonfounder mutations identified. The mutation prevalence among patients without FC/AJ ancestry was 4.9% (95% CI, 2.6% to 8.8%). Mutations were more frequent in patients diagnosed at ≤ 50 years of age ($P = .03$) and in patients with either two or more first- or second-degree relatives with pancreas, breast, ovarian or prostate cancer, or one such relative and a second primary of one of these cancer types ($P < .001$). *BRCA1*, *BRCA2* and *PALB2* carriers with late-stage (III or IV) disease had an overall survival advantage ($P = .049$), particularly if treated with platinum-based chemotherapies ($P = .030$).

CONCLUSION: Considering these results, we recommend reflex founder mutation testing of patients with FC/AJ ancestry and full gene sequencing of patients who are ≤ 50 years or meet the identified family history criteria. Reflex testing of all incident patients for these four genes may become justified as full gene sequencing costs decline.

3.3 INTRODUCTION

Pancreatic adenocarcinoma (PAC) remains a lethal malignancy.[339,340,345] Although novel combinations of systemic therapies have been evaluated in PAC over the past decade,[5,365] these efforts have not resulted in marked improvements in patient survival. These clinical failures,

together with the projection that PAC will be the second leading cause of cancer death by 2030,[15] highlight the urgent need for both early detection and precision therapy strategies for PAC.

Genomic subtypes of PAC have been described and may inform therapies. PAC associated with defective homology-directed DNA repair (HDR), frequently attributable to germline mutations in *BRCA1*, *BRCA2*, or *PALB2*, exhibits genomic instability that is characterized by large numbers of single-nucleotide variants, short deletions, and structural variations.[37,296] Importantly, evidence is accumulating that HDR-deficient tumors have increased sensitivity to platinum-based DNA cross-linking agents and poly(ADP-ribose) polymerase inhibitors (PARPis),[37,303,311,314] which may improve clinical outcomes.[317]

Previous studies have estimated a *BRCA1* and *BRCA2* germline mutation prevalence of 3% to 5% in patients with incident PAC.[134,135] Although mutations in *PALB2* have been reported in up to 3% of patients with familial PAC, these seem to be extremely rare among patients with incident PAC in North America.[134,135,142,145] The contribution of these genes may be more substantial in populations enriched with recurrent (i.e., founder) mutations, like the Ashkenazi Jewish (AJ) and French-Canadian (FC) populations.[180,182,366] Three founder mutations in *BRCA1* and *BRCA2* are known in the AJ population, with a reported prevalence of 5.5% to 12.1% among patients with incident PAC.[129,134] In the FC population, 20 founder mutations in *BRCA1*, *BRCA2*, and *PALB2* have been reported that play an important role in hereditary breast/ovarian cancer,[195-198,200,201,344] although their prevalence among patients with PAC has not been reported. *ATM* germline mutations have been reported in 2.4% to 3.2% of patients with familial PAC and 1.7% to 4.2% of unselected patients with PAC.[135,136,142,147] Although involved in the HDR pathway, *ATM*-associated PAC rarely shares the genomic features of PAC with BRCA1, BRCA2 or PALB2 deficiency,[37,296] and the therapeutic sensitivities of ATM-deficient PAC remain unknown. In this

large, two-center study, we assessed whether reflex genetic testing of *BRCA1, BRCA2, PALB2*, and *ATM* should be offered to all or select patents with incident PAC.

3.4 METHODS

3.4.1 Study Participants

Two case series were evaluated. The FC Study participants included consecutively enrolled patients with a clinical diagnosis of PAC and self-reported FC ancestry (one or more grandparents) enrolled in the Quebec Pancreas Cancer Study (QPCS)[364] between April 2012 and June 2017. Patients who were self-referred or referred from medical genetics were excluded. The Montreal-Toronto (MT) Study participants included patients, unselected for ancestry or family history, with a clinical diagnosis of PAC who were consecutively enrolled in the QPCS or Ontario Pancreas Cancer Study[333] between August 2014 and December 2015. DNA from circulating lymphocytes, saliva, and/or tissue specimens was obtained from participants and their relatives for germline genetic testing, segregation, and/or somatic studies (Data Supplement). Ethics approval was obtained for both studies.

Clinical variables and family history data were extracted from the QPCS and Ontario Pancreas Cancer Study registries.[333,364] Eligibility for clinical genetic testing was assessed using the National Comprehensive Cancer Network (NCCN) BRCA-Related Breast and/or Ovarian Cancer Syndrome Testing Criteria v2.2016.[367] For the NCCN criteria, a second primary cancer in the proband was considered equivalent to a cancer in a close blood relative, and all prostate cancers were assumed to have a Gleason score < 7, because these were unavailable.

3.4.2 FC Founder Mutation Screening

Screening for the 20 FC founder mutations (11 *BRCA1*, eight *BRCA2* and one *PALB2*; listed with historical names in Data Supplement)[195-198,200,344] was performed on a rolling basis. Screening was performed by polymerase chain reaction and bead-based fluorescent detection on the Luminex 200 platform[198,368] or by Sanger sequencing (primers listed in Data Supplement).

3.4.3 Full Gene Sequencing

In the FC Study, full gene sequencing of *BRCA1*, *BRCA2*, *PALB2*, and *ATM* was performed after founder mutation testing (Data Supplement). MT Study participants underwent panel testing, which included full *BRCA1*, *BRCA2*, *PALB2*, and *ATM* gene sequencing (Data Supplement).

All protein-truncating variants (nonsense, frameshift, or canonical splice-site) were presumed to be pathogenic, with the exception of the low-penetrance *BRCA2*:c.9976A>T (p.Lys3326*) variant.[369] Variants were validated by Sanger sequencing. Synonymous and nonsynonymous single-nucleotide variants were further evaluated if annotated as pathogenic or likely pathogenic in ClinVar.[370] Because variants of unknown significance (VUS) are not well characterized in the FC founder population, rare (minor allele frequency < 1% in public databases)[371-373] nonsynonymous single-nucleotide and noncanonical splice-site variants were evaluated in the FC Study case series. These VUS were assessed for pathogenicity using in silico prediction algorithms (Data Supplement).

3.4.4 Multiplex Ligation-Dependent Probe Amplification

Patients with PAC with FC ancestry have not previously been characterized for mutations in these four genes. Therefore, FC Study participants were evaluated for large insertions/deletions or rearrangements in *BRCA1*, *BRCA2*, *PALB2*, and *ATM* by multiplex ligation-dependent probe

amplification (MLPA; Data Supplement).

3.4.5 Mutation Carriers

If samples were available, pathogenic variants and VUS predicted pathogenic were assessed for segregation in relatives affected with PAC or associated cancers and for loss-of-heterozygosity (LOH) or somatic silencing of the wild-type allele in the tumors (Data Supplement).

3.4.6 Statistical Analysis

Categorical and continuous patient variables were compared using Fisher's exact test or *t* test, respectively. Associations with overall survival (OS) were tested by log-rank (GraphPad Prism v6.0, La Jolla, California). For the OS analyses, *ATM* carriers were included in the non-carrier group as it remains uncertain whether these patients exhibit similar genomic and therapeutic profiles as *BRCA1*, *BRCA2*, and *PALB2* carriers. OS was defined as the time from clinical diagnosis of PAC to death by any cause or date of last follow up. Date of clinical diagnosis was considered the date of first documentation of radiological evidence of PAC. If imaging reports were unavailable, date of biopsy or surgery was used. FC patients that underwent founder testing only were not considered in statistical analyses, because nonfounder mutations could not be excluded.

3.5 RESULTS

One hundred seventy-four patients underwent reflex FC founder mutation testing during the study period. A clinical or pathologic diagnosis of PAC and FC ancestry was confirmed in 150

patients. The first 114 of these patients also underwent full gene sequencing of *BRCA1*, *BRCA2*, *PALB2*, and *ATM* to identify nonfounder mutations. Eight of 150 (5.3%; 95% CI, 2.6% to 10.3%) patients carried a founder mutation, and 3 of 114 (2.6%; 95% CI, 0.6% to 7.8%) patients tested across the four genes carried nonfounder mutations, including one mutation in *ATM*, for which there are no known FC founder mutations (Table 3.1). Among the 114 patients tested for both founder and nonfounder mutations, the total mutation prevalence was 7.0% (95% CI, 3.4% to 13.4%; Table 3.1). Notably, an FC patient who was excluded from our analyses because of a final pathologic diagnosis of ampullary carcinoma was found to carry an *ATM* mutation (Table 3.2). Thirty-one VUS were identified, of which six were either predicted pathogenic or to affect splicing (Data Supplement). Finally, no large genomic changes in the four genes were observed in the first 90 patients with PAC tested. Considering these results, MLPA was performed only for *BRCA1* and *BRCA2* for the subsequent 24 samples, because large alterations are more common in these genes,[204,374-376] with no mutations identified.

Two-hundred ninety-one patients were enrolled during the MT Study period. After excluding 44 patients who overlapped with the FC Study and 11 patients with a final diagnosis that was not PAC, 236 patients remained. Three of 30 patients with PAC reporting AJ ancestry were found to carry founder mutations (10%; 95% CI, 2.7% to 26.4%; Table 3.1). Of the remaining 206 patients with PAC without FC or AJ (nonfounder) ancestry, 10 mutations were identified (4.9%; 95% CI, 2.6% to 8.8%; Table 3.1). Table 3.2 shows mutation details as well as clinical and family history variables for the individual carriers.

The results from segregation and somatic silencing analyses of mutation and VUS carriers are described in the Data Supplement. The pedigrees of the *PALB2* mutation carriers demonstrate cosegregation of the germline mutation with PAC in family FC-2 (Fig. 3.1A) and a PAC

phenocopy in family FC-58 (Fig. 3.1B). The pedigrees for the remaining FC Study carriers are shown in the Data Supplement.

Clinicopathologic characteristics, including responses to chemotherapy of all carriers, are shown in Table 3.2, with additional mutation details in the Data Supplement. The clinical courses of mutation carriers in the FC Study are described in the Data Supplement. Clinical and family history variables were compared between carriers and noncarriers (Table 3.3) and stratified by ancestry in the Data Supplement. Carriers were diagnosed with PAC at a younger age compared with noncarriers (P = .03). Five of 31 (16.1%) patients with PAC diagnosed at age 50 years or younger were carriers (P = .03), with a sensitivity and specificity of 23.8% (95% CI, 8.22% to 47.2%) and 92.1% (95% CI, 88.6% to 94.8%), respectively. A family history (one or more first- or second-degree relatives) of PAC (P = .03), breast cancer (P = .02), or prostate cancer (P = .009) were each significantly associated with carrier status. Considering these cancers together, probands with PAC and at least two first- or second-degree relatives with pancreas, breast, ovarian or prostate cancer on the same side of the family, or one such relative and a second primary of one of these cancer types, were significantly more likely to be carriers (P < .001), with a sensitivity of 66.7% (95% CI, 43.0% to 85.4%) and specificity of 82.4% (95% CI, 77.8% to 86.4%). Carriers were also more likely to meet NCCN guidelines for genetic testing compared with noncarriers (P = .006), with a sensitivity of 61.9% (95% CI, 38.4% to 81.9%) and specificity of 70.1% (95% CI, 64.8% to 75.0%).

Of the patients included in the OS analyses, 99 patients were alive and 251 were deceased at the time of last follow-up (median, 309 days; range, 0 to 1,611 days). Median OS was 789 and 431 days for carriers versus noncarriers, respectively. A trend toward improved OS in carriers was observed for all stages combined (hazard ratio [HR], 0.561; 95% CI, 0.388 to 1.03; P = .068; Fig.

3.2A). For late stage alone (III or IV), mutation carriers had improved OS compared to noncarriers (Fig. 3.2B; HR, 0.521; 95% CI, 0.360 to 0.991; P = .049). This difference was more significant when carriers who did not receive platinum-based therapies were excluded (Fig. 3.2C; HR, 0.449; 95% CI, 0.32 to 0.94; P = .030).

3.6 DISCUSSION

We have assessed the clinical utility of reflex genetic testing of *BRCA1*, *BRCA2*, *PALB2*, and *ATM* by evaluating 386 patients with PAC. A previous study of patients with incident PAC reported a *BRCA1* and *BRCA2* mutation prevalence of 4.6%.[134] This observation was driven by a 12.1% founder mutation prevalence among AJ patients, while the prevalence among non-AJ patients was 3.7%.[134] Hu *et al.* reported a similar prevalence of 3.1% among 96 unselected patients with PAC without AJ ancestry.[135] Consistent with these reports, we observed a *BRCA1* and *BRCA2* mutation prevalence of 3.4% (95% CI, 1.5% to 7.0%) among nonfounder cases and an AJ founder mutation prevalence of 10% (95% CI, 2.7% to 26.4%).

It is well established that individuals with FC ancestry in North America have an excess of distinct Mendelian diseases.[180,182] Although the province of Quebec encompasses the largest population of FC descendants, FCs populate across Canada and in regions of the United States, particularly in New England and the Midwestern United States.[183,377] Up to 10% of New England states' residents reported FC ancestry in a 2000 census,[183] which may contribute to the higher prevalence of disease-causing alleles in these states.[378] In this study, we identified an FC founder mutation prevalence of 5.3% (95% CI, 2.6% to 10.3%) among consecutive patients with PAC and FC ancestry, whereas only two of 114 patients tested for mutations in *BRCA1, BRCA2*, and *PALB2* carried nonfounder mutations. Moreover, one of the two rare nonfounder mutations identified did

not originate from the FC branch of the patient's pedigree, whereas the second nonfounder mutation was identified in an adopted patient where paternal ancestry could not be confirmed. Although we did not identify any *BRCA1* mutations, pathogenic mutations in *BRCA1* are less common than in *BRCA2* in PAC, and our study is not sufficiently powered to exclude a contribution of *BRCA1* mutations.[126,134,154] Considering the low cost of targeted mutation screening, we recommend founder mutation testing include all 20 FC founder mutations.

In the FC Study case series, two carriers had the *PALB2* c.2323C>T (p.Gln775*) FC founder mutation. In contrast, no *PALB2* mutations were identified in the MT Study, which was unselected for ancestry. Since Jones *et al*[137] first reported *PALB2* as a PAC predisposition gene, observed in 3.1% of patients who are at high-risk, subsequent studies have reported the prevalence of *PALB2* mutations in PAC to be much lower (0 to 0.6%) in North America.[126,134,142,145,154] Our observation that 1.3% (95% CI, 0.1% to 5.0%) of FC patients carried the *PALB2* c.2323C>T FC founder mutation suggests that *PALB2* may have a greater role in FCs with PAC. The observation of cosegregation with disease, as well as LOH of the wild-type allele in the tumors of affected carriers in family FC-2 provides further support of a causal role for this *PALB2* mutation in PAC.

Among patients with PAC with FC ancestry, a single pathogenic *ATM* mutation was identified (0.9%; 95% CI, 0% to 5.3%), which has only been previously reported as a somatic variant.[379] An additional germline *ATM* mutation, with LOH in the tumor, was identified in an FC patient with a final diagnosis of ampullary carcinoma. A recent genomic study of ampullary carcinomas described mutations in *ATM*; however, it was not indicated whether these mutations originated in the germline.[380] No *ATM* mutations were identified among the patients reporting AJ ancestry. Among nonfounder patients, three *ATM* mutations were identified (1.5%; 95% CI, 0.3% to 4.4%), consistent with the 4.2% prevalence among unselected patients with PAC previously

reported.[135]

No large genomic changes in *BRCA1*, *BRCA2*, *PALB2*, or *ATM* were identified in our FC Study case series. Consistent with these findings are reports showing absence of large genomic changes in *BRCA1, BRCA2*, and/or *PALB2* in families with breast/ovarian cancer with FC ancestry,[196,204,381,382] AJ ancestry,[383] and ethnically diverse families.[134,376,384,385] Large genomic changes in *ATM* are also rare.[386] Collectively, these studies suggest that MLPA analyses for these four genes in patients with incident PAC should not be necessary.

In recent years, numerous studies have demonstrated that BRCA1- and BRCA2-deficient PAC tumors are sensitive to platinum-based chemotherapies or PARPis.[37,303,311,314] Although less well established, similar findings in PALB2-deficient tumors treated with DNA damaging agents have been reported.[316,387] Here, we report two patients with germline *PALB2* mutations treated with platinum-based chemotherapy. One patient had a partial response and the second patient had a 95% pathologic tumor regression. Much less is known about the clinical behavior of patients with PAC with *ATM* mutations, and the genomes of ATM-deficient PAC tumors infrequently exhibit the genomic hallmarks associated with HDR deficiency.[37,296] Although there is some evidence that ATM-deficient breast and prostate cancers have sensitivity to olaparib (a PARPi),[330,331] the chemotherapeutic sensitivities to platinum-based agents and PARPis in ATM-deficient PAC remains unknown. In our study, one *ATM* carrier was treated with FOLFIRINOX (leucovorin, fluorouracil [5-FU], irinotecan, and oxaliplatin), and had disease stability but no pathologic tumor response.

Our OS observations are in keeping with a previous retrospective study demonstrating superior OS for late-stage *BRCA1* and *BRCA2* carriers treated with platinums.[317] We observed a survival advantage for *BRCA1*, *BRCA2*, and *PALB2* carriers with late stage (III and IV) disease

that was more pronounced when excluding carriers that were not treated with platinum-based therapies. Although this was not an intent-to-treat study, the carrier status of participants may have influenced chemotherapy choices as well as decisions to include surgical and ablative approaches to manage advanced disease. Because these treatment decisions were not uniform, and in the absence of intent-to-treat clinical trial data, it is not possible to fully assess the benefit of platinum therapy in carriers.

Although our study was focused on HDR genes, additional genetic subtypes of PAC may be amenable to precision oncology strategies. The genomes of PAC tumors with deficiency in one or more of the DNA mismatch repair genes (*MLH1*, *MSH2*, *MSH6*, and *PMS2*) exhibit increased numbers of mutation-associated neoantigens[296] and favorable responses to immunotherapy.[388] Although genetic studies have reported the prevalence of germline mutations in these genes among patients with incident PAC to be low (0% to 2%),[135,142] these studies have not considered large genomic changes (e.g., exon deletions), which are prevalent in patients with Lynch syndrome.[99] Considering the promise of immunotherapy, genetic studies to fully assess the prevalence of mismatch repair germline mutations in PAC are merited.

Finally, although age at diagnosis and family history have not consistently been shown to be predictors of *BRCA1* or *BRCA2* carrier status in PAC,[82,134] significant associations were observed in our study. Nearly one in six patients with PAC diagnosed at age 50 years or younger were found to be carriers of a mutation in one of the four HDR genes. We found that patients with PAC meeting family history criteria of two or more first- or second-degree relatives with pancreas, breast, ovarian or prostate cancer on the same side of the family, or one such relative and a second primary of one of these cancer types, identified carriers with similar sensitivity and specificity to the NCCN guidelines. These criteria may serve as simpler screening guidelines that can be easily

applied in an ambulatory setting by oncologists and surgeons.

Considering the 4.9% mutation prevalence among consecutive patients with PAC without founder (FC/AJ) ancestry and the moderate sensitivity of genetic screening guidelines for PAC, reflex full-gene sequencing of all patients with incident PAC by oncologists and surgeons, with referral of only mutation-positive patients to medical genetics, may become justified as full gene sequencing costs decline and precision oncology strategies for carriers become the standard of care. In the interim, in view of existing healthcare systems and considering our observed founder mutation frequencies, we recommend reflex founder mutation testing of patients with incident PAC with FC or AJ ancestry, and reflex full-gene sequencing for these four HDR genes in patients without founder ancestry or who test negative for founder mutations who are diagnosed at age 50 years or younger or who meet our described family history criteria. The implementation of these recommendations for patients with FC and perhaps other[216] founder population ancestry, as well as for patients with young onset of disease or a family history, may lead to improved clinical outcomes and may have preventative and early-detection cancer screening implications for relatives found to be carriers.

3.7 ACKNOWLEDGEMENTS

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Fellowship (C.W.). G.Z. is a clinical research scholar of the Fonds de recherche du Québec – Santé.

3.8 FIGURES

Figure 3.1. Pedigrees of the two families with *PALB2*:c.2323C>T (p.Gln775*) mutations. A) Family FC-2. B) Family FC-58. Carrier status is depicted for all the patients in whom germline DNA was available and tested. +/- indicates heterozygous carrier status. +/+ indicates wild type. Probands are indicated with an arrow. Individuals shaded in black are affected with pancreatic adenocarcinoma, and individuals shaded in grey are affected with a tumor other than pancreatic adenocarcinoma. The ages of living family members and the ages of death (d.) for deceased individuals are indicated in years. Tumor types and ages at diagnosis are indicated in years.

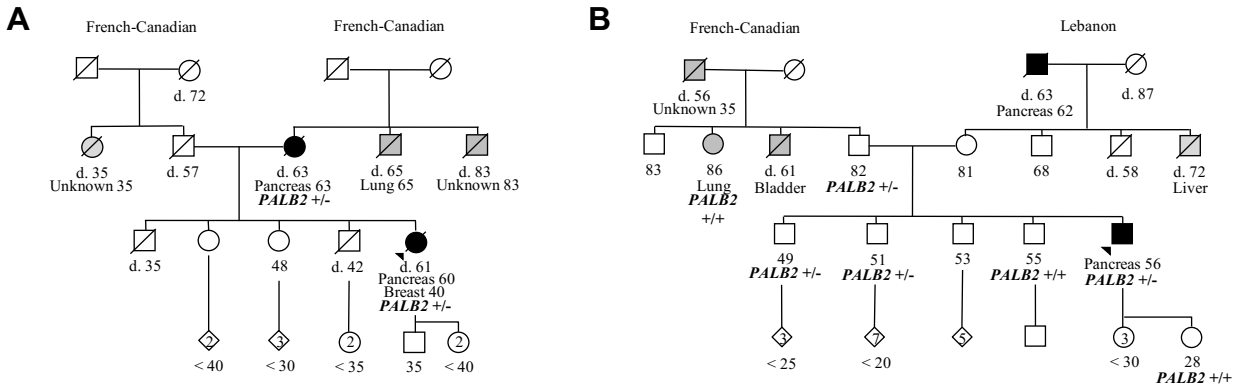
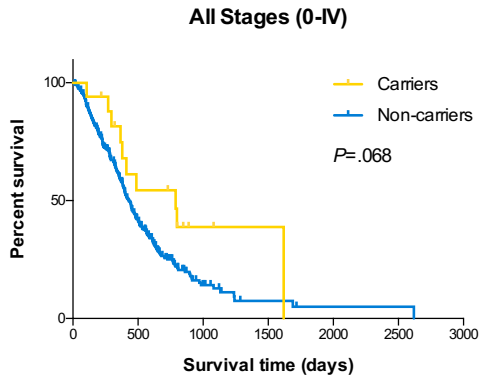


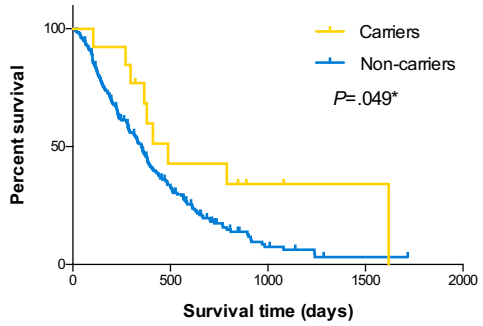
Figure 3.2. Kaplan-Meier survival curves for (A) all stages (0-IV) carriers versus noncarriers, (B) late-stage (III/IV) carriers versus noncarriers, and (C) late-stage (III/IV) carriers treated with platinum versus noncarriers. *ATM* mutation carriers were considered noncarriers for these analyses. Log-rank *P* values are indicated. OS, overall survival.

A



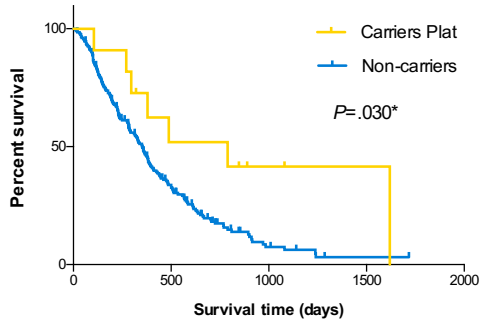
No. at risk:						
Carriers	17	8	2	1		
Non-carriers	331	116	13	3	1	1

B



No. at risk:				
Carriers	13	5	2	1
Non-carriers	205	60	7	1

C



No. at risk:				
Carriers	11	5	2	1
Non-carriers	205	60	7	1

3.9 TABLES

Table 3.1. Pathogenic Mutation Prevalence.

	French-Canadian Study (n=150)						Montreal-Toronto Study (n=236)					
	Founder panel testing (n=150)			Full gene testing (n=114*)			Ashkenazi Jewish (n=30)			Non-Founder† (n=206)		
	No.	%	CI (95%)	No.	%	CI (95%)	No.	%	CI (95%)	No.	%	CI (95%)
BRCA2	6	4.0	1.7 - 8.6	2	1.8	0 - 6.6	2	6.7	0.8 - 22.4	6	2.9	1.2 - 6.4
BRCA1	0	0	0 - 3.4	0	0	0 - 3.9	1	3.3	0 - 18.1	1	0.5	0 - 3.0
PALB2	2	1.3	0.1 – 5.0	0	0	0 - 3.9	0	0	0 - 13.5	0	0	0 - 2.2
ATM	0	0	0 - 3.4	1	0.9	0 - 5.3	0	0	0 - 13.5	3	1.5	0.3 - 4.4
All 4 HDR genes												
Founder mutations	8	5.3	2.6 - 10.3	-	-	-	3	10	2.7 - 26.4	-	-	-
Non-founder mutations	-	-	-	3‡	2.6‡	0.6 - 7.8	0	0	0 - 13.5	10	4.9	2.6 - 8.8
Total	8	5.3	2.6 - 10.3	3	2.6	0.6 – 7.8	3	10	2.7 - 26.4	10	4.9	2.6 - 8.8

Abbreviation: HDR, homology-directed DNA repair.
*Results shown only for nonfounder mutations identified by full gene sequencing and multiplex ligation-dependent probe amplification and exclude 36 French-Canadian patients tested only for founder mutations.
†No self-reported Ashkenazi Jewish or French-Canadian ancestry.
‡This includes a mutation in *ATM*, for which there are no known French-Canadian founder mutations.

Table 3.2. Pathogenic Mutation Carriers.

ID	Gene	Variant	Sex	Age Dx (yrs)	Stage	Surgery	Chemotherapy	Response to Chemo		Vital Status	OS (days)	PHx Cancer, age dx	NCCN
								Radiologic	Pathologic				
FC-14	*BRCA2*	**c.3170_3174delAGAAA (p.Lys1057Thrfs*8)**	M	47	IV	Distal pancreatectomy, RFA of liver mets	FOLFIRINOX	PR	50% regression	D	1619	-	Y
FC-137*	*BRCA2*	**c.3170_3174delAGAAA (p.Lys1057Thrfs*8)**	M	77	IIA	-	Gemcitabine/ nab-paclitaxel	SD	-	A	480	-	Y
FC-109	*BRCA2*	**c.3545_3546delTT (p.Phe1182*)**	M	74	IV	-	Gemcitabine/ nab-paclitaxel	PD	-	A	321	-	N
							Gemcitabine/ cisplatin	PR	-				
FC-62	*BRCA2*	c.4171G>T (p.Glu1391*)	F	50	IV	-	Gemcitabine, FOLFIRINOX	MR	-	D	379	-	Y
FC-18	*BRCA2*	c.4691dupC (p.Thr1566Aspfs*9)	M	47	III	Total pancreatectomy	FOLFIRINOX, gemcitabine/ cisplatin	PR	75% regression	D	789	-	Y
FC-125*	*BRCA2*	**c.8537_8538delAG (p.Glu2846Glyfs*22)**	M	37	IIB	-	Gemcitabine, FOLFIRINOX, FOLFIRI	PR	-	A	364	-	N
FC-148*	*BRCA2*	**c.8537_8538delAG (p.Glu2846Glyfs*22)**	M	49	IIA	-	FOLFIRINOX	SD	-	A	268	-	Y
FC-93	*BRCA2*	**c.9004G>A (p.Glu3002Lys)**	F	61	III	Pancreaticoduodenectomy	FOLFIRINOX	CR	100% regression	A	889	-	Y
FC-2	*PALB2*	**c.2323C>T (p.Gln775*)**	F	60	III	-	FOLFIRINOX, gemcitabine/ cisplatin	PR	-	D	296	Br, 40	Y
FC-58	*PALB2*	**c.2323C>T (p.Gln775*)**	M	56	III	Total pancreatectomy	FOLFIRINOX, gemcitabine	PR	95% regression	A	1080	-	N
FC-95	*ATM*	c.3802delG (p.Val1268*)	M	46	III	Pancreaticoduodenectomy	Gemcitabine, FOLFIRINOX	SD	No response	A	582	-	Y
FC-115[†]	*ATM*	c.748C>T (p.Arg250*)	F	74	III	Pancreaticoduodenectomy	-	-	-	D	335	Br, 45	Y
AJ-4	*BRCA2*	[‡]**c.5946delT (Ser1982Argfs*22)**	F	68	IV	-	Gemcitabine/ nab-paclitaxel	MR	-	D	410	Thy, 24	Y
AJ-13	*BRCA2*	[‡]**c.6174delT (Ser1982Argfs*22)**	M	51	IIA	Pancreaticoduodenectomy	Gemcitabine/ cisplatin	-	-	A	729	-	Y
AJ-25	*BRCA1*	[§]**c.68_69delAG (p.Glu23Valfs*17)**	M	76	III	-	Gemcitabine/ cisplatin	PR	-	D	488	Mel, 54, 74; Pro, 68	Y
NF-96	*BRCA2*	c.658_659delGT (p.Val220Ilefs*4)	M	72	IV	-	Gemcitabine/ cisplatin/ veliparib	SD	-	D	271	-	N
NF-17	*BRCA2*	c.927delA (p.Leu310Tyrfs*14)	F	53	IIB	-	FOLFIRINOX	R	-	D	799	-	N
NF-148	*BRCA2*	[‖]**c.3109C>T (p.Gln1037*)**	M	29	IV	-	FOLFIRINOX	R	-	D	105	-	Y
NF-187	*BRCA2*	c.5065_5066delGCinsAAA p.(Ala1689Lysfs*6)	M	76	I	Pancreaticoduodenectomy	-	-	-	D	217	-	Y
NF-123	*BRCA2*	c.7008-2A>T and [#]c.631G>A (p.Val211Ile)	M	79	IV	-	Gemcitabine/ nab-paclitaxel	MR	-	D	366	Br, 67; Pro, 76	Y
NF-175	*BRCA2*	c.8677C>T (p.Gln2893*)	F	61	IIB	Pancreaticoduodenectomy	Gemcitabine/ oxaliplatin	-	-	A	800	-	Y
							Gemcitabine/ cisplatin	-	-				
NF-7	*BRCA1*	c.2125_2126insA (p.Phe709Tyrfs*3)	F	54	IV	-	FOLFIRINOX	R	-	A	847	-	N
NF-67	*ATM*	c.3033_3034insAGGGATGCTCAAGGACTGTGAACACC (p.Gln1017Leufs*2)	M	59	IIA	Pancreaticoduodenectomy	Gemcitabine	-	-	A	772	-	N
NF-72	*ATM*	**c.2250G>A (p.Lys750=)	M	67	IB or IIB	Pancreaticoduodenectomy	Gemcitabine	-	-	A	418	-	N
NF-111	*ATM*	c.5188C>T (p.Arg1730*)	M	74	I-III	Pancreaticoduodenectomy	Gemcitabine	-	-	A	625	Sar, 73; Crc, 74	N

NOTE. Founder mutations are bolded. Meeting NCCN genetic testing criteria is indicated.

Abbreviations: A, alive; AJ, Ashkenazi Jewish; Br, breast; CR, complete response; Crc, colorectal; D, deceased; Dx, diagnosis; FC, French Canadian; FOLFIRI, leucovorin, fluorouracil (5-FU), irinotecan; FOLFIRINOX, folinic acid (leucovorin), fluorouracil (5-FU), irinotecan, oxaliplatin; Mel, melanoma; MR, mixed response; N, no; NAB-paclitaxel, nanoparticle albumin-bound paclitaxel; NCCN, National Comprehensive Cancer Network; OS, overall survival; PD, progressive disease; PHx, personal histry; PR, partial response (>30%); Pro, prostate; R, response (<30%), RFA, radiofrequency ablation; SD, stable disease; Thy, thyroid; Sar, sarcoma; Y, yes.

This mutation carrier was identified among the case series screened only for FC founder mutations and is therefore excluded from statistical analyses.

[†]This patient's final pathology revealed ampullary carcinoma.

[‡]This AJ founder mutation is historically known as c.6174delT.

[§]This AJ founder mutation is historically known as c.185delAG.

[‖]This *BRCA2* mutation is a known Southern Chinese founder mutation, although it was identified in a patient reporting Indian ancestry.[389]

[#]This variant results in the loss of splice donor site for intron 7 and is known to co-occur with the c.7008-2A>T mutation.[370]

**This synonymous variant in *ATM* is annotated in ClinVar as pathogenic because it has been shown to disrupt a splice donor site and has been associated with ataxia-telangiectasia.[370] Exon skipping in the tumor of NF-72 was confirmed by whole transcriptome sequencing (Data Supplement).

Table 3.3. Clinical and family history characteristics of patients with PAC.

	Carriers (n=21)		Non-carriers (n=329)		
	No.	%	No.	%	*P*
Age, years					
Mean	60.2		65.2		**.03***
Range	29-79		35-92		
					.03*
≤50	5	23.8	26	7.9	
>50	16	76.2	303	92.1	
Gender					.38
Male	14	66.7	185	56.2	
Female	7	33.3	144	43.8	
Smoking History					.64
Yes	12	63.2	158	57	
No	7	36.8	119	43	
Diabetes					.18
Yes	5	33.3	39	18.7	
No	10	66.7	170	81.3	
Pancreatitis					.18
Yes	2	13.3	10	4.6	
No	13	86.7	207	95.4	
Stage					.64
Early	6	30	117	36.4	
Late	14	70	204	63.6	
Resection					.06
Yes	11	52.4	104	31.7	
No	10	47.6	224	68.3	
Personal cancer history					
>1 primary ca	4	19	59	17.9	1
Breast	2	9.5	10	3	.15
Ovarian	0	0	2	0.6	1
Prostate	2	9.5	8	2.4	.12
Family history					
≥ 1 FDR/SDR w/ breast ca	11	52.4	90	27.8	**.02***
≥ 1 FDR/SDR w/ ovarian ca	3	14.3	16	4.9	.10
≥ 1 FDR/SDR w/ prostate ca	9	42.9	57	17.6	**.009***
≥ 1 FDR/SDR w/ PAC	7	33.3	45	13.9	**.03***
≥ 2 FDR/SDR* breast, ovarian, prostate ca or PAC	14	66.7	57	17.6	**<.0001***
Criteria for genetic testing					
Met NCCN guidelines	13	61.9	97	29.5	**.006***

NOTE. Values are indicated as No. (%) unless otherwise noted. *P* values < .05 indicate significance. These analyses exclude 36 French-Canadian patients tested only for founder mutations. Data Supplement presents these data stratified by ancestry (French-Canadian, Ashkenazi Jewish, and nonfounder).
Abbreviations: FDR, first-degree relative; NCCN, National Comprehensive Cancer Network; PAC, pancreatic adenocarcinoma; SDR, second-degree relative.
*Two or more relatives on the same side of the family; a second primary of one of these cancer types was considered equivalent to a relative.

3.10 SUPPLEMENTAL METHODS

Full Gene Sequencing

Genomic DNA was extracted from peripheral blood lymphocytes using organic solvent isolation (phenol:chloroform) or using the Qiagen Puregene DNA extraction kit (Qiagen, Hilden, Germany). Saliva DNA was isolated according to the manufacturer's instructions (Oragene Saliva Kit, DNA Genotek Inc.).

For the French-Canadian (FC) Study, all 23 coding exons (exons 2 – 24) of *BRCA1* (NM_007294.3), all 26 coding exons (exons 2 – 27) of *BRCA2* (NM_000059.3), all 13 coding exons (exons 1 – 13) of *PALB2* (NM_024675.3), and all 62 coding exons (exons 2 – 63) of *ATM* (NM_058216.2) plus 20 bp from the exon boundaries were amplified in 81, 134, 54, and 183 amplicons, respectively, using Wafergene SmartChip technology (Wafergene Inc, California, USA). Illumina next-generation sequencing adaptors and sample unique DNA barcodes were incorporated into the amplicons with a second PCR. The prepared DNA libraries were pooled and paired-ends were sequenced at 2x250 cycles on the Illumina MiSeq sequencer.

For the Montreal-Toronto (MT) Study, a custom gene panel was created using the Agilent SureSelect technology (Agilent Technologies, Santa Clara, California, USA), which captured the coding exons (plus 10 bp flanking intronic regions) of *BRCA1*, *BRCA2*, *PALB2* and *ATM*. Briefly, germline DNA samples were sheared (200-400bp) and Illumina adaptors were added to their ends. For each sample, the DNA fragments were barcoded by tagging a specific oligonucleotide to all DNA fragments of that sample. The regions of interest from each sample DNA library were captured from the rest of the genome by hybridizing them with biotinylated RNA strand probes which were complementary to the DNA sequence of the regions of interest. Binding of the hybridized DNAs with biotinylated probes to streptavidin-coated magnetic beads allowed

separation from the remaining genomic DNA in a magnetic field. Every 16 samples were pooled for paired-end sequencing (600 cycles, generating 300 bp reads) on an Illumina MiSeq, using a V3 sequencing cartridge (Illumina Inc., San Diego, California, USA).

For both the FC and MT Studies, the sequence reads for each DNA sample were aligned to the reference sequences of the four genes using the Burrows-Wheeler Aligner.[390] The Picard package was used to convert the SAM files to BAM format, and to sort and index the BAM files.[391] Next, all the unmapped reads or reads with low mapping quality as well as reads aligned to more than one region were filtered out from the BAM file using the GATK package.[392] The UnifiedGenotyper module of the GATK package was used for the FC Study, while the HaplotypeCaller module of GATK was used for the MT Study, to call single nucleotide variants and insertions/deletions. Variants were considered if they had at least twenty-fold depth of coverage and the alternate allele was present in at least 25% of the reads. All pathogenic variants identified were confirmed using Sanger sequencing.

Variants of Unknown Significance (VUS)

Since variants of unknown significance (VUS) are not well characterized in the FC founder population, rare (minor allele frequency <1% in public databases)[371-373] nonsynonymous single nucleotide and non-canonical splice-site variants were evaluated in the FC Study case series (Table S5). These VUS were assessed for pathogenicity using 4 *in silico* prediction algorithms: SIFT (score <0.05)[393], PolyPhen 2 (score >0.909)[394], GERP (score >2)[395] and CADD (c score >15).[396] Non-canonical splice-site variants (splice acceptor/donor variants >±2 bp and intron-exon junction variants) were assessed using two *in silico* splicing prediction algorithms: Human Splice Finder (score >65 is considered to be a functional splice site, and any mutation that results in loss/creation

of a splice site with a score difference >10% between wild-type and mutant sequences are predicted to affect splicing)[397] and MaxEntScan (score >3 is considered to be a functional splice site, and any mutation that results in loss/creation of a splice site with a score difference >20% between wild-type and mutant sequences are predicted to affect splicing)[398]. Rare synonymous (silent) variants identified by full gene sequencing were also searched in ClinVar[370] for clinical associations, as synonymous variants have previously been implicated in disease.[399,400]

Multiplex Ligation-Dependent Probe Amplification (MLPA)

For the FC Study case series, MLPA was performed using lymphocyte or saliva genomic DNA extracted as described above. Probe sets P002 (*BRCA1*), P090 (*BRCA2*), P260 (*PALB2*) and P041&P042 (*ATM*) from MRC-Holland (Amsterdam, the Netherlands) were used according to the manufacturer's instructions. Probes were hybridized to the genomic DNA and then adjacent probe pairs were ligated and amplified. Fragment analysis of the amplified probes was performed on an ABI 3500XL DNA analyzer (Applied Biosystems). GeneMarker software (SoftGenetics LLC., PA, USA) was used for calling large insertions/deletions.

Mutation Carriers

Carriers of pathogenic variants and VUS predicted pathogenic were further characterized by segregation analyses in families with a history of pancreatic adenocarcinoma (PAC) or related tumor spectrum cancers (e.g., breast). Genomic DNA from lymphocytes, saliva or archived formalin-fixed paraffin-embedded (FFPE) tissue samples of affected family members was tested for segregation by Sanger sequencing. Lymphocyte and saliva genomic DNA was isolated as

described above, while genomic DNA from FFPE was extracted using the QIAamp DNA FFPE Tissue kit (Qiagen, Hilden, Germany).

In affected cases, where FFPE tumor tissue was available, tumor DNA was extracted and tested for loss-of-heterozgygosity (LOH) of the wild type allele by Sanger sequencing. Regions of tumor cellularity >50% were macrodissected prior to DNA extraction. LOH was assessed by visually comparing allelic ratios of paired tumor and normal tissue DNA.

Whole Genome Sequencing and Whole Transcriptome Sequencing

For patient NF-72, tumor DNA and RNA were extracted from fresh frozen tissue samples following laser capture microdissection (LCM). Whole genome and whole transcriptome sequencing was then performed as described elsewhere.[296] As a reference, germline DNA was extracted from circulating lymphocytes for whole genome sequencing.

Briefly, frozen tissue was sectioned by cryotome into 8-µm thick sections, lightly stained with hematoxylin, and LCM was performed on a Leica LMD 7000 instrument on the same day to minimize nucleic acid degradation, and stored at -80°C until further processing. Qiagen Cell Lysis Buffer and Arcturus PicoPure Extraction Buffer were used for genomic DNA and RNA extractions, respectively. RNA was isolated following LCM using the PicoPure RNA Isolation Kit (Thermo Fisher, Waltham, MA), then DNase treated using an RNase-free DNase Set (Qiagen, Valencia, CA).

For whole genome sequencing of normal and tumor DNA, Illumina paired-end libraries were prepared using the KAPA Library Preparation Kits (KAPA Biosystems, Woburn, MA USA Cat#KK8230), quantified on the Illumina Eco Real-Time PCR Instrument (Illumina Inc., San Diego, CA, USA) using KAPA Illumina Library Quantification Kits (KAPA Biosciences,

Woburn, MA, USA Cat#KK4835), all according to the manufacturer's protocols. Paired-end cluster generation and sequencing was carried out on the Illumina HiSeq 2000/2500 platform.

Germline variants were called using the Genome Analysis Tool Kit (GATK4, version 1.3.16), following GATK "best practices". Somatic single nucleotide variations (SNVs) were identified as the intersection of calls by two Strelka 5 version 1.0.7 and MuTect6 version 1.1.14, both run using default settings. Somatic indels were identified by Strelka5. Tumor ploidy, cellularity, loss of heterozygosity and copy number segments were called using an in-house algorithm.[45] Somatic structural rearrangements were identified as the union of calls from CREST14 version alpha and DELLY15 version 0.5.5.

Tumor RNA libraries were prepared using the TruSeq RNA Access Library Sample prep kit (Illumina Inc., San Diego, CA) and quantified on the Illumina Eco RealTime PCR Instrument (Illumina) using KAPA Illumina Library Quantification Kits (KAPA Biosciences, Woburn, MA) all according to manufacturer's protocols. Sequencing was carried out on the Illumina HiSeq 2500 platform. Reads were aligned to the human genome (hg19) and transcriptome (Ensembl v75) using STAR v.2.4.2a22. Picard v.1.12123 marked duplicated reads. Gene expression was calculated in fragments per kilobase of exon per million reads mapped (FPKM) using cufflinks package v.2.2.124.

Germline and tumor whole genome and tumor transcriptome aligned reads were visualized with the Integrative Genomics Viewer.[401]

3.11 SUPPLEMENTAL RESULTS

Segregation and Somatic Silencing Analyses

Segregation of the *PALB2* c.2323C>T mutation with PAC was observed in the family of proband FC-2 (Fig. 1A, main text), as well as LOH in the proband's previous breast cancer and her mother's liver metastasis. In the family of patient FC-58, the *PALB2* c.2323C>T mutation was found to originate from the paternal branch of the family with FC ancestry, suggesting that the patient's maternal grandfather with PAC was a phenocopy (Fig. 1B, main text). The non-founder *BRCA2* mutation identified in patient FC-62 was determined to originate from the non-FC branch of the family (Figure S1). Of the 5 *BRCA2* carriers that were assessed, two exhibited inactivation of the wildtype allele in the corresponding tumors (Table S4). Notably, LOH of the *ATM*:c.2250G>A (p.Lys750=) mutation was observed in the tumor of NF-72 by whole genome sequencing, with skipping of exon 14 resulting in an in-frame deletion seen by whole transcriptome sequencing (Figure S2). LOH was not identified in a second *ATM* carrier (FC-74) (Table S4), but was observed in the tumor of the *ATM* carrier with ampullary cancer (FC-115). Family history was either unremarkable or samples were not available for the remaining carriers.

Detailed Clinical Histories of FC Study Mutation Carriers

Patient FC-14 with the *BRCA2*:c.3170_3174delAGAAA (p.Lys1057Thrfs*8) mutation presented with stage IV disease but underwent a distal pancreatectomy and splenectomy following complete response (CR) of his liver metastases and partial response (PR; >30%) of his primary tumor. He also had radiofrequency ablation of two liver metastases identified by intraoperative ultrasound. Although patients with stage IV disease are not surgical candidates, this patient's favorable disease evolution on FOLFIRINOX and his inability to continue on chemotherapy due

to thrombocytopenia were listed as the basis for offering the patient surgery. Postoperatively, the patient continued to be treated with both systemic therapy (FOLFIRINOX) and unconventional local-regional approaches. He eventually succumbed to his disease at approximately 4.5 years from diagnosis.

Patient FC-109 with the *BRCA2*:c.3545_3546delTT (p.Phe1182*) mutation presented with stage IV disease and started on gemcitabine/nab-paclitaxel as first line for metastatic PAC prior to the availability of his genetic testing results. Following disease progression (PD) with 8 cycles of gemcitabine/nab-paclitaxel, the *BRCA2* germline mutation status was considered and the patient was switched to gemcitabine/cisplatin with PR (>30%) following 4 cycles. He remains alive on palliative gemcitabine/cisplatin.

Patient FC-18 with the *BRCA2*:c.4691dupC (p.Thr1566Aspfs*9) presented with stage III PAC and underwent systemic therapy with FOLFIRINOX followed by gemcitabine/cisplatin (7 cycles). As there was PR (>30%) of his primary tumor and no evidence of metastatic disease with excellent performance on therapy, the patient had external beam radiation (25 Gy) to his primary tumor followed by a curative intent resection. He underwent a total pancreatectomy with splenectomy and *en bloc* vascular resection (portal vein and superior mesenteric artery). He completed an adjuvant course of FOLFIRINOX, but eventually developed disease recurrence, progression and death.

Patient FC-93 with the *BRCA2*:c.9004G>A (p.Glu3002Lys) mutation was treated with FOLFIRINOX following her presentation with a locally advanced tumor (stage III). She had CR with neoadjuvant therapy and underwent a pancreaticoduodenectomy with no residual tumor in the resection specimen. She remains disease-free.

Patient FC-2 with the *PALB2*:c.2323C>T (p.Gln775*) presented with stage III disease and was treated with FOLFIRINOX. She did not tolerate FOLFIRINOX, and was switched to gemcitabine/cisplatin with PR (>30%) of her primary tumor and no evidence of distant disease. She subsequently underwent surgical exploration and was found to have a sub-centimetric liver metastasis. She did not return to systemic therapy, her disease progressed, and she passed away.

Patient FC-58 with the *PALB2*:c.2323C>T (p.Gln775*) presented with stage III disease and underwent a neoadjuvant course of FOLFIRINOX with a PR (>30%) of his tumor and excellent performance on therapy. Following a subsequent course of external beam radiation (25 Gy) to the primary tumor, he underwent a curative intent *en bloc* total pancreatectomy, splenectomy, right hemicolectomy and superior mesenteric vein resection. He completed an adjuvant course of gemcitabine and he is currently disease-free.

Patient FC-95 with the *ATM*:c.3802delG (p.Val1268*) presented with stage III disease and underwent a neoadjuvant course of Gemcitabine (2 cycles), FOLFIRINOX (7 cycles) and external beam radiation (25 Gy), followed by a pancreaticoduodenectomy. His pathology showed no response to neoadjuvant treatment. The patient then underwent an adjuvant course of FOLFIRINOX (4 cycles) and palliative gemcitabine (3 cycles), with disease stability, and was alive at the time of data collection.

Patient FC-125 with the *BRCA2*:c.8537_8538delAG (p.Glu2846Glyfs*22) mutation presented with stage IIB disease. After 8 cycles of FOLFIRINOX, imaging showed stable disease. He was switched to FOLFIRI due to peripheral neuropathy and remains alive.

Patient FC-62 with the *BRCA2*:c.4171G>T (p.Glu1391*) mutation presented with stage IV disease with multiple liver metastases. She underwent palliative chemotherapy with 1 cycle of gemcitabine with PD and was switched to FOLFIRINOX. Although she had PR (>30%) of the

primary tumor and the liver metastases following 8 cycles of FOLFIRINOX, new bone lesions appeared (PD). She succumbed to her disease one year following her diagnosis.

Patient FC-137 with the *BRCA2*:c.3170_3174delAGAAA (p.Lys1057Thrfs*8) mutation presented with stage IIA disease and he was deemed unresectable at the time of diagnosis due to tumor involvement of the superior mesenteric vein and poor performance status. The patient went on to have 12 cycles of gemcitabine/nab-paclitaxel with disease stability. He was then referred for a second surgical opinion at the McGill University Health Centre, enrolled in the QPCS, and found to carry a mutation in *BRCA2*. Consequently, a recommendation was made to the treating oncologist to consider gemcitabine/cisplatin.

Patient FC-148 with the *BRCA2*:c.8537_8538delAG (p.Glu2846Glyfs*22) mutation presented with stage IIA disease. His tumor was considered unresectable at the time of diagnosis due to involvement of the superior mesenteric vein. He went on to be treated with 6 cycles of FOLFIRINOX with disease stability (SD) but received a surgical opinion that he remained unresectable. Considering his disease stability, the patient decided against further treatment with chemotherapy and he remained off chemotherapy for 5 months before being referred to the MUHC for a second surgical opinion. He was subsequently enrolled in the QPCS and the *BRCA2* mutation was identified. He currently is being restaged for resection consideration.

3.12 SUPPLEMENTAL FIGURES

Figure S3.1. Pedigrees of FC pathogenic mutation carriers. Carrier status is depicted for all the cases in which germline DNA was available and tested. +/- indicates heterozygous carrier status. +/+ indicates wild-type. Probands are indicated with an arrow. Individuals shaded in black are affected with PAC, while individuals shaded in grey are affected with a tumor other than PAC. The ages of living family members and the ages of death (d.) for deceased individuals are indicated in years. Tumor types and ages at diagnosis are indicated in years. Abbreviations: CRC, colorectal. N.B. The pedigrees of the two *PALB2* mutation carriers are shown in the main text (Figure 3.1).

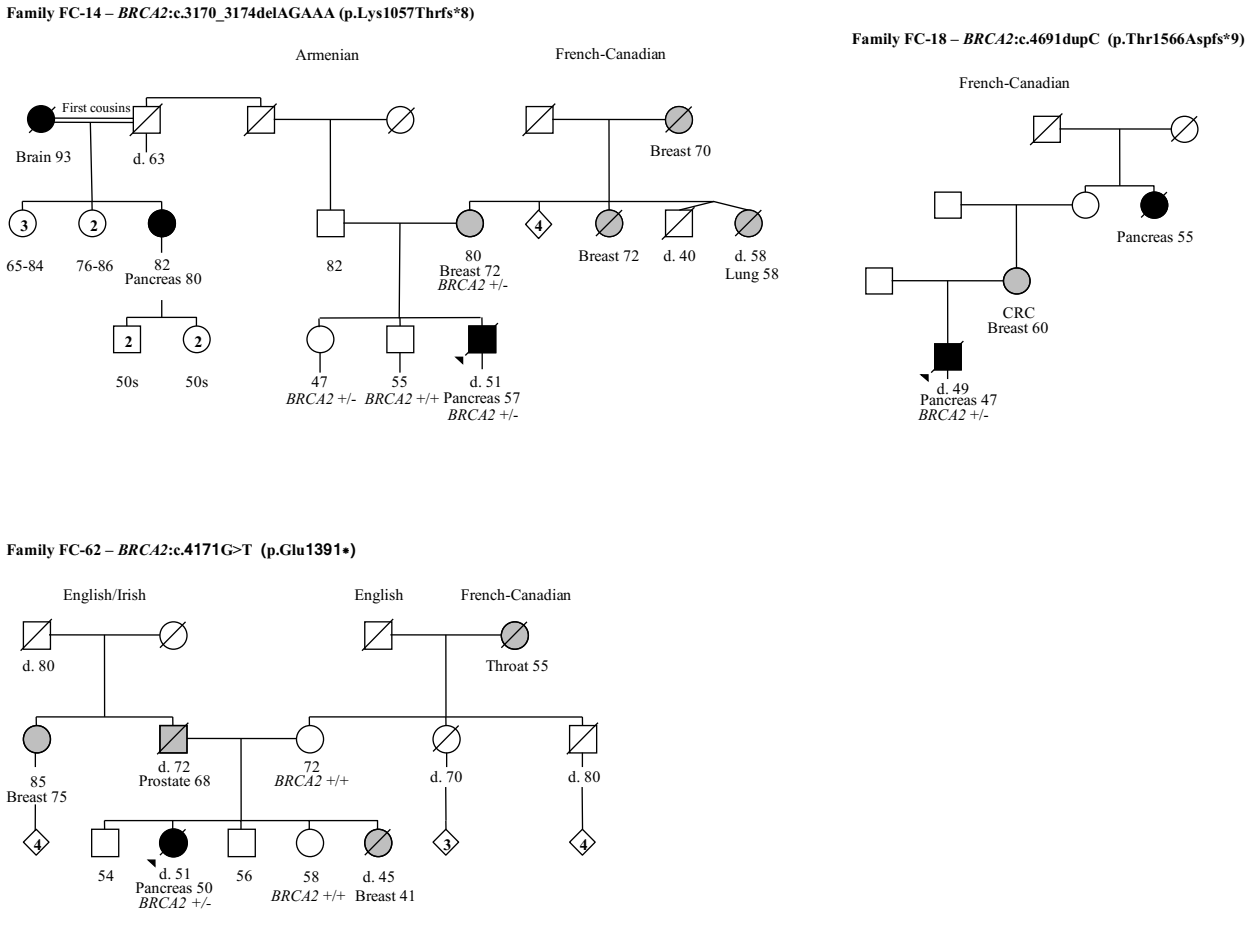
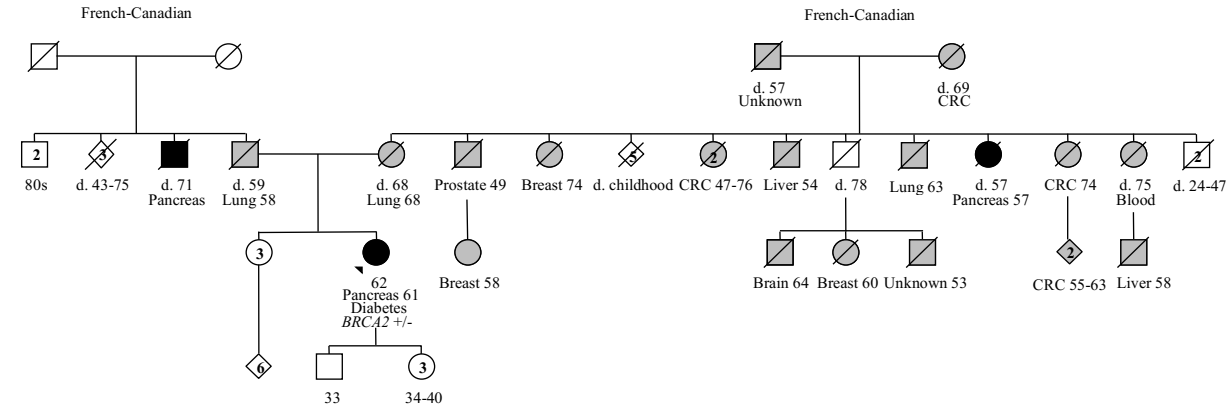
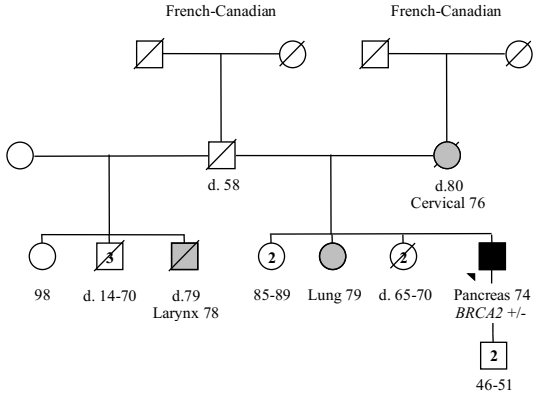


Figure S3.1 (continued).

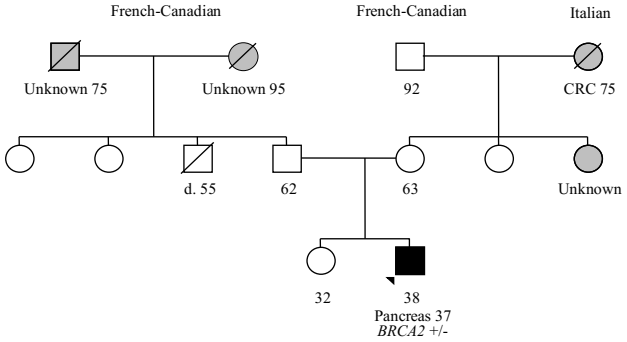
Family FC-93 – *BRCA2*:c.9004G>A (p.Glu3002Lys)



Family FC-109 - *BRCA2*:c.3545_3546delTT (p.Phe1182*)



Family FC-125 – *BRCA2*:c.8537_8538delAG (p.Glu2846Glyfs*22)



Family FC-95 - *ATM*:c.3802delG (p.Val1268*)

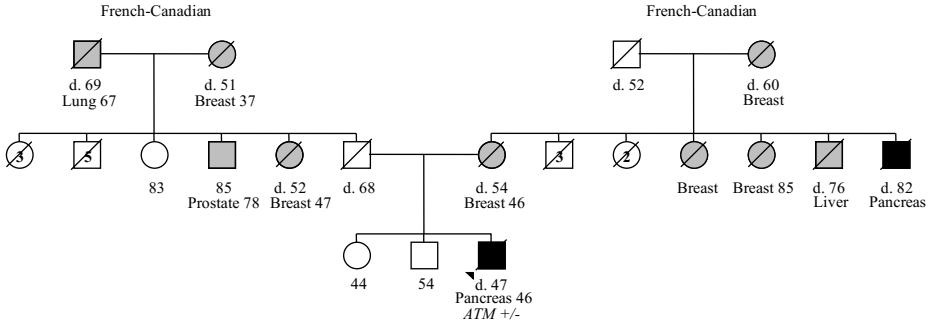
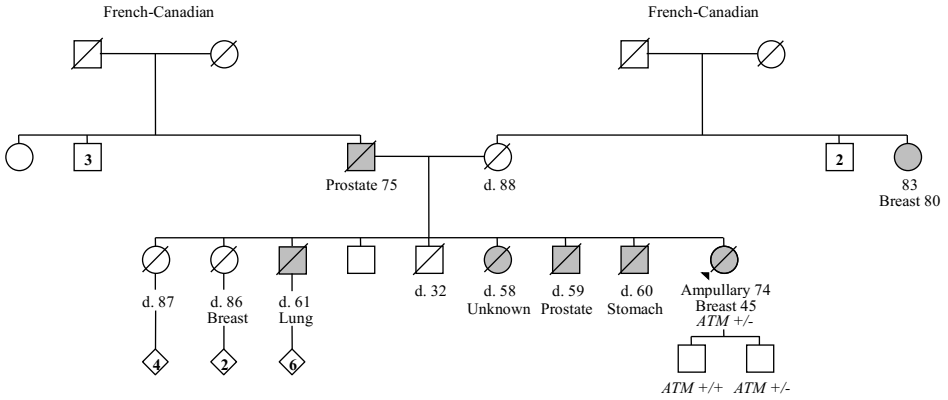
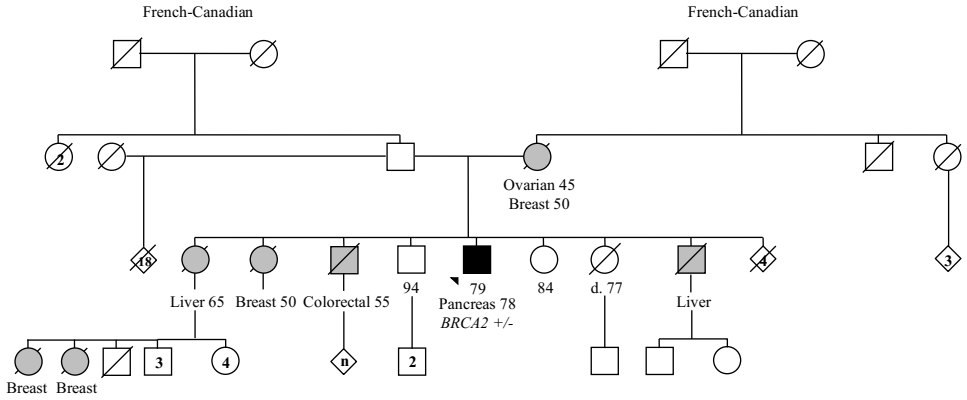


Figure S3.1 (continued).

Family FC-115 – *ATM*:c.748C>T (p.Arg250*)



Family FC-137 - *BRCA2*:c.3170_3174delAGAAA (p.Lys1057Thrfs*8)



Family FC-148 - *BRCA2*:c.8537_8538delAG (p.Glu2846Glyfs*22)

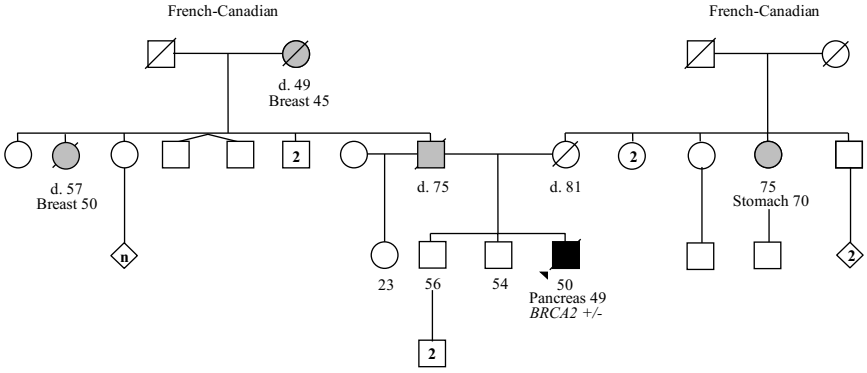
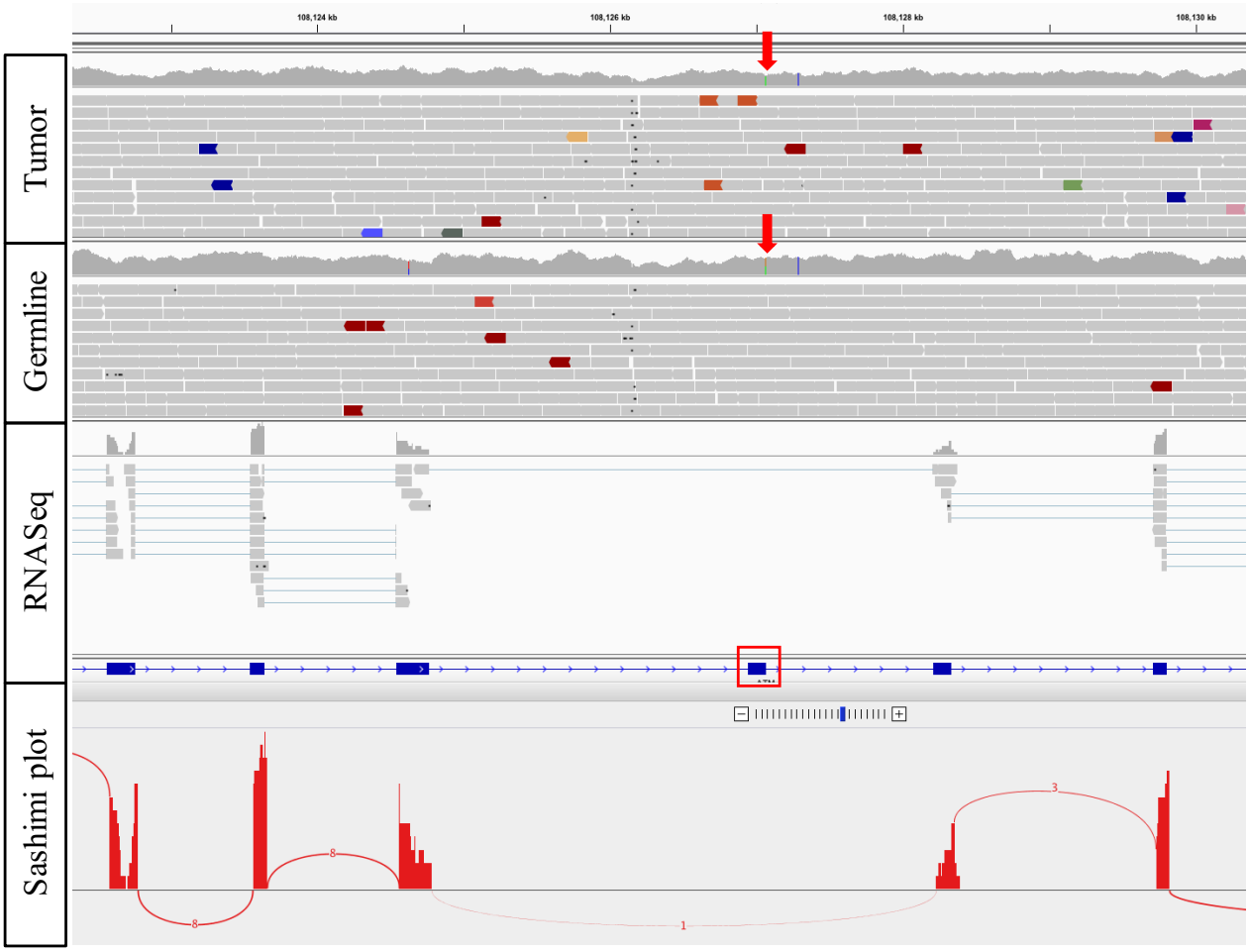


Figure S3.2. LOH of the *ATM*:c.2250G>A (p.Lys750=) mutation with complete exon 14 skipping in patient NF-72's tumor genome. Tumor, germline and RNASeq reads from the Integrative Genomics Viewer are shown, with Sashimi plot below. The variant is marked with a red arrow, and is heterozygous in the germline and homozygous in the tumor. Exon 14 of the reference sequence is indicated by the red box, and is absent from the RNASeq reads and Sashimi plot.



3.13 SUPPLEMENTAL TABLES

Table S3.1. List of the 20 known French-Canadian founder mutations in *BRCA1*, *BRCA2* and *PALB2*.

Gene	Variant (HGVS nomenclature)	Other Names	Chr Pos (GRCh37)	Exon	rsID	FHx PAC	References
BRCA1 (NM_007294.3)	c.962G>A (p.Trp321Ter)	G1081A; W321X	Chr17:41246586	11	rs80357292		[194,195,198,199,201]
	c.1016dupA (p.Val340Glyfs*6)	1129insA; 1135insA; Val340fs	Chr17:41246532	11	rs80357569		[192,197]
	c.1054G>T (p.Glu352Ter)	G1173T; E352X	Chr17:41246494	11	rs80357472		[197,199]
	c.1961dupA (p.Tyr655Valfs*18)	1961_1962insA; 2080_2081insA; 2080insA	Chr17:41245587	11	rs80357853		[198,199]
	c.2125_2126insA (p.Phe709Tyrfs*3)	2244insA	Chr17:41245422	11	rs80357871		[196,198,199]
	c.2834_2836delGTAinsC (p.Ser945Thrfs*6)	2953del3+C; 2953GTAinsC; 950ter	Chr17:41244712	11	rs386134270		[184,186,187,189,194,195,197-199,201]
	c.3649_3650insA (p.Ser1217Tyrfs*2)	3768insA; Ser1217fs; 1218ter	Chr17:41243898	11	rs80357831		[186,187,189,194,196,198,199,201]
	c.3756_3759delGTCT (p.Ser1253Argfs*10)	3875delGTCT; 3874del4; 3875del4; Ser1253fs	Chr17:41243789	11	rs80357868		[194,195,197-199,201]
	c.4327C>T (p.Arg1443*)	C4446T; R1443X; Arg1443X	Chr17: 41234451	13	rs41293455	Yes[189]	[186,187,189,191,194-199,201]
	c.5102_5103delTG (p.Leu1701Glnfs*14)	5221delTG	Chr17: 41215940	18	rs80357608		[189,194,196,198,199,201]
	c.5536C>T (p.Gln1846*)	Q1846X	Chr17: 41197751	24	rs80356873		[196,198]
BRCA2 (NM_000059.3)	c.2588dupA (p.Asn863Lysfs*18)	2816insA; 880ter; Asn863fs; c.2588dupA; 2588_2589insA	Chr13: 32911080	11	rs606231399		[186,187,189,194,196,198,199,201]
	c.2808_2811delACAA (p.Ala938Profs*22)	2806_2809delAAAC; 3034delAAAC; 3034del4; 3036delACAA	Chr13: 32911300	11	rs80359352		[194,196,198,199,201]
	c.3170_3174delAGAAA (p.Lys1057Thrfs*8)	3398delAGAAA;3398del5;Lys1057fs	Chr13: 32911662	11	rs80359373	Yes[193]	[193-199,201]
	c.3545_3546delTT (p.Phe1182*)	3773delTT;Phe1182X	Chr13: 32912037	11	rs80359388		[194,196,198,199,201]
	c.5857G>T (p.Glu1953*)	G6085T;E1953X;Glu1953X	Chr13: 32914349	11	rs80358814		[186-189,194-199,201]
	c.6275_6276delTT (p.Leu2092Profs*7)	2099ter; 6503delTT;Leu2092fs	Chr13: 32914767	11	rs11571658		[186-189,194,196,198,199,201]
	c.8537_8538delAG (p.Glu2846Glyfs*22)	8765delAG; 2867ter; 8535delAG	Chr13: 32945142	20	rs80359714	Yes[185,186,189]	[185-190,194-199,201]
	c.9004G>A (p.Glu3002Lys)	E3002K;Glu3002Lys	Chr13: 32953937	23	rs80359152	Yes[200]	[196,198,200]
PALB2 (NM_024675.3)	**c.2323C>T (p.Gln775*)**	Q775X	Chr16: 23641152	5	rs180177111		[195,204,344]

Abbreviations: HGVS, Human Genome Variation Server; Chr Pos, chromosomal position; FHx, family history; PAC, pancreatic adenocarcinoma. Mutations identified in this study are bolded.

Table S3.2. Primers used for PCR of 20 French-Canadian founder mutations.

Gene	Variant	Forward Primer	Reverse Primer	Amplicon Size (bp)
BRCA1 (NM_007294.3)	c.962G>A (p.Trp321Ter)	TCCCCATCATGTGAGTCATC	GAGCCATGTGGCACAAATAC	394
	c.1016dupA (p.Val340Glyfs*6)	CATCAGCTACTTTGGCATTTG	CCAGCTCATTACAGCATGAGA	400
	c.1054G>T (p.Glu352Ter)	CATCAGCTACTTTGGCATTTG	CCAGCTCATTACAGCATGAGA	400
	c.1961dupA (p.Tyr655Valfs*18)	CCTGAGTGCCATAATCAGTACC	AAGCTGAACCTATAAGCAGCAGT	594
	c.2125_2126insA (p.Phe709Tyrfs*3)	CCTGAGTGCCATAATCAGTACC	AAGCTGAACCTATAAGCAGCAGT	594
	c.2834_2836delGTAinsC (p.Ser945Thrfs*6)	TTCCCATTTCTCTTTCAGGTG	ACATTCTCTGCCCACTCTGG	399
	c.3649_3650insA (p.Ser1217Tyrfs*2)	TGCACTGTGAAGAAAACAAGC	TGCATCTCAGGTTTGTTCTGA	481
	c.3756_3759delGTCT (p.Ser1253Argfs*10)	TGCACTGTGAAGAAAACAAGC	TGCATCTCAGGTTTGTTCTGA	481
	c.4327C>T (p.Arg1443*)	GAGCAGGGACAAGAACCAAG	CATGGGCATTAATTGCATGA	500
	c.5102_5103delTG (p.Leu1701Glnfs*14)	CCCAGCATCACCAGCTTATC	GTCACCAGGGGTTTTAGAATCA	400
	c.5536C>T (p.Gln1846*)	CCAAGGGAGACTTCAAGCAG	TGCTTGTGTTCTCTGTCTCCA	348
BRCA2 (NM_000059.3)	c.2588dupA (p.Asn863Lysfs*18)	TCATGAAAATGCCAGCACTC	GAGTTCTTGAAAATGGGTTCG	491
	c.2808_2811delACAA (p.Ala938Profs*22)	GCTGTTGCCACCTGAAAAAT	CCTAAGAGTCCTGCCCATTTG	500
	c.3170_3174delAGAAA (p.Lys1057Thrfs*8)	TGTTCTTGCAGAGGAGAACAA	CTGGTTTTCAGGCACTTCAA	600
	c.3545_3546delTT (p.Phe1182*)	ACCTAGCCAAAAGGCAGAAA	TGAGCAGAATAAAAGCCCCTA	357
	c.5857G>T (p.Glu1953*)	GCACGCATTCACATAAGGTTT	TGAGCTGGTCTGAATGTTCG	338
	c.6275_6276delTT (p.Leu2092Profs*7)	CATTCAGACCAGCTCACAAGA	AAGCCTGTTCTTTTCCCAAA	496
	c.8537_8538delAG (p.Glu2846Glyfs*22)	GAGGAAATGTTGGTTGTGTTGA	TGATATTTCTGTCCCTTGTTGC	786
	c.9004G>A (p.Glu3002Lys)	CCACTACTAATGCCCACAAAGA	GGTTTGTACCGGTAGTTGTTGA	291
PALB2 (NM_024675.3)	c.2323C>T (p.Gln775*)	AGCTCCTGGCATGTGTTTCT	CACCTGCTTTCCCCATCTTA	321

Table S3.3. Description of the 31 variants of unknown significance (VUS) validated by Sanger sequencing, including population frequencies, predicted pathogenicity scores, loss of heterozygosity (LOH) status and cancer family history.

Gene	Chr Pos (GRCh37)	Variant (HGVS nomenclature)	rsID	MAF EVS	MAF 1000G	MAF ExAC	SIFT	PolyPhen-2	CADD	GERP	HSF			MaxEntScan			ClinVar	ID	LOH	FHx cancer
											WT	Mut	%	WT	Mut	%				
ATM	Chr11:108098576	c.146C>G (p.Ser49Cys)	rs1800054	0.0099	0.0055	0.0074	0.00	0.98	25.10	4.22	.			.			benign	FC-17	.	1 3DR br
ATM	Chr11:108117691	c.902G>A (p.Gly301Asp)	rs202208861	7.70E-05	0	4.12E-5	0.00	0.99	23.80	4.98	90.6	87.5	-3.46	7.6	6.9	-9.57	.	FC-82	.	1 FDR ov
ATM	Chr11:108117787	c.998C>T (p.Ser333Phe)	rs28904919	0.00138	0.0014	0.00128	0.01	0.35	15.90	3.85	.			.			likely benign	FC-111	.	1 2DR br, 1 2DR pro
ATM	Chr11:108124761	c.2119T>C (p.Ser707Pro)	rs4986761	0.0085	0.005	0.0078	0.26	0.00	15.81	3.81	.			.			likely benign	FC-5, FC-109	.	.
ATM	Chr11:108128232	c.2275A>G (p.Ser759Gly)	rs148705269	0.00023	0	2.5E-5	0.15	0.72	13.39	3.73	.			.			uncertain significance	FC-36	.	3 FDR br, 2 2DR br, 1 FDR pro
ATM	Chr11:108128246	c.2289T>A (p.Phe763Leu)	rs34231402	0.00046	0.00046	0.00051	0.33	0.00	16.81	5.03	.			.			uncertain significance	FC-26	.	.
ATM	Chr11:108138003	c.2572T>C (p.Phe858Leu)	rs1800056	0.009	0.0069	0.0091	0.13	0.73	8.82	2.85	.			.			benign	FC-22	.	PHx pro
ATM	Chr11:108153437	c.3577G>A (p.Val1193Ile)	rs779148780	0	0	8.24E-6	0.13	0.44	14.14	5.38	.			.			uncertain significance	FC-58		1 2DR PAC
ATM	Chr11:108170491	c.5056A>G (p.Ile1686Val)	rs145453814	0.00015	0	2.5E-5	0.20	0.68	19.23	5.20	.			.			uncertain significance	FC-15	.	1 2DR br, 1 2DR pro
ATM	Chr11:108173677	c.5417T>C (p.Ile1806Thr)	rs773546064	0	0	8.24E-6	0.11	0.89	22.10	5.27	.			.			uncertain significance	FC-51	.	1 FDR br
ATM	Chr11:108175463	c.5558A>T (p.Asp1853Val)	rs1801673	0.0048	0.0027	0.0052	0.02	0.88	23.60	5.52	.			.			benign	FC-104	.	1 2DR PAC, 1 3DR br, 1 FDR pro
BRCA1	Chr17:41256153	c.427G>A (p.Glu143Lys)	rs80356991	0	0	1.6E-5	0.01	0.02	20.80	3.28	.			.			benign	FC-19	.	PHx blad
BRCA1	Chr17:41246190	c.1358A>C (p.Glu453Ala)	.	0	0	0	0.01	0.84	7.35	3.48	.			.			.	FC-110	.	1 FDR br
BRCA1	Chr17:41245027	c.2521C>T (p.Arg841Trp)	rs1800709	0.0024	0.0014	0.0017	0.00	0.00	19.95	2.84	.			.			likely benign	FC-23	.	1 2DR br, 1 3DR br
BRCA1	Chr17:41244757	c.2791G>T (p.Val931Leu)	rs763639161	0	0	8.24E-6	0.07	0.06	9.25	1.75	.			.			uncertain significance	FC-81	.	.
BRCA1	Chr17:41243840	*c.3708T>G (p.Asn1236Lys)	rs28897687	0.00023	0.00046	0.00024	0.00	0.03	0.07	-2.85	.			.			uncertain significance	FC-43	.	PHx br, 1 3DR br
BRCA1	Chr17:41243509	*c.4039A>G (p.Arg1347Gly)	rs28897689	0.0048	0.00092	0.004	0.01	0.26	18.95	2.98	.			.			uncertain significance	FC-39	.	.
BRCA1	Chr17:41215366	c.5177G>T (p.Arg1726Ile)	rs786203547	0	0	0	0.02	0.09	22.80	3.45	.			.			uncertain significance	FC-53	.	.
BRCA2	Chr13:32907395	c.1780A>T (p.Ile594Leu)	rs431825287	0	0	0	0.16	0.60	20.50	-5.34	.			.			uncertain significance	FC-81	.	.
BRCA2	Chr13:32911929	c.3437A>G (p.Glu1146Gly)	rs80358588	0	0	8.24E-6	0.25	0.01	4.99	-1.31	.			.			uncertain significance	FC-110	.	1 FDR br

BRCA2	Chr13:32914815	*c.6323G>A (p.Arg2108His)	rs35029074	0.00023	0.00092	0.0013	0.27	0.35	13.20	2.76	.			.			benign	FC-37	.	.
BRCA2	Chr13:32920979	c.6953G>A (p.Arg2318Gln)	rs80358921	0	0	1.64E-5	0.01	1.00	24.20	5.03	.			.			uncertain significance	FC-105	.	.
BRCA2	Chr13:32930669	c.7540A>G (p.Lys2514Glu)	rs864622624	0	0	0	0.01	0.99	29.70	5.48	.			.			uncertain significance	FC-103	.	.
BRCA2	Chr13:32953464	c.8765G>A (p.Ser2922Asn)	rs730881567	0	0	0	0.05	0.94	21.30	4.73	.			.			uncertain significance	FC-103	.	.
BRCA2	Chr13:32953971	c.9038C>T (p.Thr3013Ile)	rs28897755	0.0004616	0	0.0002306	0.10	0.21	14.78	3.91	.			.			benign	FC-114	.	.
BRCA2	Chr13:32969073	c.9501+3A>T	rs61757642	0.000231	0	0.000148	.	.	.	.	92.5	84.8	-8.37	10.3	4.4	-57.6 BD	uncertain significance	FC-103	.	.
BRCA2	Chr13:32972626	c.9976A>T (p.Lys3326*)	rs11571833	0.00646	0.00439	0.0070096	.	.	.	.	.			.			benign	FC-72, FC-103	.	PHx chol†, 1 FDR br‡, 1 FDR pro‡; N/A
PALB2	Chr16:23649273	c.109C>T (p.Arg37Cys)	rs200048921	0	0	8.24E-6	0.00	1.00	29.10	5.70	45.7	72.5	+58.7 CD	10.1	9.8	-2.39	uncertain significance	FC-43	No	PHx br, 1 3DR br
PALB2	Chr16:23647211	*c.656A>G (p. Asp219Gly)	rs45594034	0.00015	0	0.00017	0.50	0.00	0.01	-5.08	.			.			uncertain significance	FC-29	.	.
PALB2	Chr16:23637715	*c.2590C>T (p.Pro864Ser)	rs45568339	0.00239	0.00183	0.00266	0.58	0.16	12.03	2.82	.			.			benign	FC-102	.	1 FDR br, 1 2DR br
PALB2	Chr16:23635370	*c.2794G>A (p.Val932Met)	rs45624036	0.00431	0.000998	0.00586	0.29	0.99	18.31	4.85	.			.			benign	FC-112	.	1 FDR PAC

Abbreviations: Chr. Pos.. chromosomal position; HGVS, Human Genome Variation Server; MAF, minor allele frequency; EVS, NHLBI Exome Variant Server; 1000G, 1000 Genomes Project; ExAC, Exome Aggregation Consortium; LOH, loss of heterozygosity; FHx, family history; PHx, personal history; PAC, pancreatic adenocarcinoma; br, breast cancer; ov, ovarian cancer; pro, prostate cancer; blad, bladder cancer; chol, cholangiocarcinoma; FDR, first-degree relative; 2DR, second-degree relative; 3DR, third-degree relative; WT, wild-type reference splicing score; Mut, mutation splicing score; BD, broken splice donor site; CD, creates new splice donor site.

Variants highlighted are either predicted pathogenic by all 4 *in silico* prediction tools (and not annotated as "benign" in ClinVar) or predicted to affect splicing by at least one *in silico* splicing prediction tool.

*Variants with asterisks have been previously reported in the FC population.[194,199,382]

†Patient presented with a synchronous hilar/Klatskin's cholangiocarcinoma.

‡These family members were known carriers of a pathogenic *BRCA1* mutation, however the proband did not carry this mutation.

Table S3.4. Description of pathogenic mutations, including population frequencies and loss of heterozygosity (LOH) status.

Gene	Chr Pos (GRCh37)	Variant (HGVS nomenclature)	Transcript ID	rsID	MAF EVS	MAF 1000G	MAF ExAC	ID	LOH
ATM	Chr11:108115560	c.708_709insA (p.Thr237Asnfs*17)	NM_000051.3	-	0	0	0	NF-177	-
ATM	Chr11:108115600	c.748C>T (p.Arg250*)	NM_000051.3	rs772821016	0	0	0.0000082	FC-115[§]	Yes
ATM	Chr11:108127067	c.2250G>A (p.Lys750=)	NM_000051.3	rs1137887	0.000077	0	0.000008	NF-72	Yes[#]
ATM	Chr11:108142089	c.3033_3034insAGGGATGCTCAAGGACTGTGAACACC (p.Gln1017Leufs*2)	NM_000051.3	-	0	0	0	NF-67	-
ATM	Chr11:108155009	c.3802delG (p.Val1268*)	NM_000051.3	rs587779834	0	0	0.000033	FC-95	No
BRCA1	Chr17:41276047	*c.68_69delAG (p.Glu23Valfs*17)	NM_007294.3	rs386833395	0	0	0.00024	AJ-25	-
BRCA1	Chr17:41245422	c.2125_2126insA (p.Phe709Tyrfs*3)	NM_007294.3	rs80357871	0	0	0	NF-7	-
BRCA2	Chr13:32900750	[†]c.631G>A (p.Val211Ile)	NM_000059.3	rs80358871	0	0	0	NF-123	-
BRCA2	Chr13:32903606	c.658_659delGT (p.Val220Ilefs*4)	NM_000059.3	rs80359604	0	0	0.000049	NF-96	-
BRCA2	Chr13:32906542	c.927delA (p.Leu310Tyrfs*14)	NM_000059.3	rs886040828	0	0	0	NF-17	-
BRCA2	Chr13:32911601	c.3109C>T (p.Gln1037*)	NM_000059.3	rs80358557	0	0	0	NF-148	-
BRCA2	Chr13:32911662	c.3170_3174delAGAAA (p.Lys1057Thrfs*8)	NM_000059.3	rs80359373	0	0	0.000025	FC-14	Yes**
BRCA2	Chr13:32912037	c.3545_3546delTT (p.Phe1182*)	NM_000059.3	rs80359388	0	0	0.000033	FC-109	Yes
BRCA2	Chr13:32912663	c.4171G>T (p.Glu1391*)	NM_000059.3	-	0	0	0	FC-62	-
BRCA2	Chr13:32913183	c.4691dupC (p.Thr1566Aspfs*9)	NM_000059.3	rs786204209	0.00008	0	0	FC-18	No
BRCA2	Chr13:32913557	c.5065_5066delGCinsAAA p.(Ala1689Lysfs*6)	NM_000059.3	rs276174852	0	0	0	NF-188	No
BRCA2	Chr13:32914666	[‡]c.5946delT (Ser1982Argfs*22)	NM_000059.3	rs786204278	0	0	0	AJ-4	-
BRCA2	Chr13:32914666	[‡]c.5946delT (Ser1982Argfs*22)	NM_000059.3	rs786204278	0	0	0	AJ-13	-
BRCA2	Chr13:32928996	c.7008-2A>T	NM_000059.3	rs81002823	0	0	0	NF-123	-
BRCA2	Chr13:32945142	c.8537_8538delAG (p.Glu2846Glyfs*22)	NM_000059.3	rs80359714	0	0	0.000008	FC-125[‖]	-
BRCA2	Chr13:32950851	c.8677C>T (p.Gln2893*)	NM_000059.3	rs397507409	0	0	0	NF-175	No
BRCA2	Chr13:32953937	c.9004G>A (p.Glu3002Lys)	NM_000059.3	rs80359152	0	0	0	FC-93	-
PALB2	Chr16:23641152	c.2323C>T (p.Gln775*)	NM_024675.3	rs180177111	0	0	0	FC-2	Yes[††]
PALB2	Chr16:23641152	c.2323C>T (p.Gln775*)	NM_024675.3	rs180177111	0	0	0	FC-58	-

Abbreviations: Chr. Pos., chromosomal position; HGVS, Human Genome Variation Server; MAF, minor allele frequency; EVS, NHLBI Exome Variant Server; 1000G, 1000 Genomes Project; ExAC, Exome Aggregation Consortium; LOH, loss of heterozygosity.

*This AJ founder mutation is historically known as c.185delAG.

[†]This variant results in the loss of splice donor site for intron 7 and is known to co-occur with the c.7008-2A>T mutation.[370]

[‡]This AJ founder mutation is historically known as c.6174delT.

[§]This patient's final pathology revealed ampullary carcinoma.

[‖]This mutation carrier was identified among the case series screened only for FC founder mutations.

[#]A somatic second hit was identified by whole genome sequencing and exon skipping was confirmed by whole transcriptome sequencing (see Supplemental Methods).

**A somatic second hit was identified in this case by whole genome sequencing and has been previously reported.[314]

[††]LOH was observed both in the proband's previous breast cancer as well as her mother's liver metastasis (PAC primary).

Table S3.5. Clinical and family history characteristics of patients with PAC stratified by ancestry.

	French-Canadian					Ashkenazi Jewish					Non-Founder‡				
	Carriers (n=8)		Non-carriers (n=106)			Carriers (n=3)		Non-carriers (n=27)			Carriers (n=10)		Non-carriers (n=196)		
	No.	%	No.	%	*P*	No.	%	No.	%	*P*	No.	%	No.	%	*P*
Age, years															
Mean	55.1		64.5		**.005***	65		68.6		.57	62.7		65.2		.51
Range	47-74		47-83			52-76		51-86			29-79		35-92		
					.001*					1					1
≤50	4	50.0	5	4.7		0	0	0	0		1	10	21	1.7	
>50	4	50.0	101	95.3		3	100	27	100		9	90	175	89.3	
Gender					1					1					.52
Male	5	62.5	65	61.3		2	66.7	13	48.1		7	70	107	54.6	
Female	3	37.5	41	38.7		1	33.3	14	51.2		3	30	89	45.4	
Smoking History					1					1.00					.75
Yes	5	83.3	48	73.8		1	33.3	12	48		6	60	98	52.4	
No	1	16.7	17	26.2		2	66.7	13	52		4	40	89	47.6	
Diabetes					.17					1					1
Yes	3	50.0	15	23.1		1	33.3	2	18.1		1	16.7	22	16.5	
No	3	50.0	50	76.9		2	66.7	9	81.8		5	83.3	111	83.5	
Pancreatitis					.42					1					.23
Yes	1	16.7	5	7.7		0	0	0	0		1	16.7	5	3.6	
No	5	83.3	60	92.3		3	100	13	100		5	83.3	134	96.4	
Stage					.056					1					.49
Early	0	0	35	33.3		1	33.3	7	26.9		5	55.6	75	39.5	
Late	8	100	70	66.7		2	66.7	19	73.1		4	44.4	115	6.5	
Resection					.13					.43					.31
Yes	5	62.5	35	33		1	33.3	4	14.8		5	50	65	33.3	
No	3	37.5	71	67		2	66.7	23	85.2		5	50	130	66.7	
Personal cancer history															
>1 primary ca	1	12.5	18	17	1	2	66.7	8	29.6	.25	1	10	33	16.8	1
Breast	1	12.5	1	0.9	.14	0	0	3	11.1	1	1	10	6	3.1	.3
Ovarian	0	0	0	0	N/A	0	0	0	0	1	0	0	2	1	1
Prostate	0	0	3	2.8	1	1	33.3	0	0	.10	1	10	5	2.6	.26
Family history															
≥ 1 FDR/SDR w/ breast ca	5	62.5	27	26.7	**.05***	1	33.3	10	37	1	5	50	53	27	.15
≥ 1 FDR/SDR w/ ovarian ca	1	12.5	7	6.9	.47	1	33.3	0	0	.10	1	10	9	4.6	.4
≥ 1 FDR/SDR w/ prostate ca	3	37.5	22	21.8	.38	1	33.3	5	18.5	.50	5	50	30	15.3	**.014***
≥ 1 FDR/SDR w/ PAC	5	62.5	15	14.9	**.005***	0	0	5	18.5	1	2	20	25	12.8	.62
≥ 2 FDR/SDR† breast, ovarian, prostate ca or PAC	6	75	23	22.8	**.004***	1	33.3	5	18.5	.50	7	70	29	14.8	**.0002***
Criteria for genetic testing															
Met NCCN guidelines	6	75	25	24.8	**.006***	3	100	27	100	1	4	40	46	23.4	0.26

Abbreviations: ca, cancer; FDR, first-degree relative; SDR, second-degree relatives; PAC, pancreatic adenocarcinoma; PHx, personal history; NCCN, National Comprehensive Cancer Network.

P values of statistical significance ($P < .05$) are bolded with an asterisk.

†2 or more relatives on the same side of the family; a second primary of one of these cancer types was considered equivalent to a relative.

‡No self-reported AJ or FC ancestry.

Chapter 4: Candidate DNA repair susceptibility genes identified by exome sequencing in high-risk pancreatic cancer

4.1 PREFACE TO CHAPTER 4

Numerous studies have demonstrated that a family history of PAC is a significant risk factor for the disease, and suggest that about 10-15% of PAC is attributable to genetic predisposition.[65,223,224] In Chapter 3, we characterized the contribution of 4 known PAC susceptibility genes (*BRCA1*, *BRCA2*, *PALB2* and *ATM*) in incident cases of PAC.[402] While mutations in these PAC susceptibility genes explain a small fraction of the familial clustering of PAC, approximately 85% of FPC remain unexplained and a major question in the field is the identification of novel genetic causes of PAC.

In the last two decades, attempts to elucidate the unexplained genetic underpinnings of FPC by traditional gene discovery methods, such as linkage analysis, have been largely unsuccessful – limited by the unavailability of samples from multiple PAC-affected family members, unknown penetrance of disease-causing alleles, presence of phenocopies and locus heterogeneity.[82]

When I joined the lab in 2012, advances in sequencing technologies had made high-throughput next-generation sequencing, particularly whole exome sequencing, widely attainable and affordable. After the discovery of two novel PAC susceptibility genes using these approaches – *PALB2* in 2009[137] and *ATM* in 2012[136] – among other cancer predisposition genes, the possibilities for gene discovery in FPC seemed limited only by the availability of biospecimens.

In Chapter 4, we generated whole exome sequencing data from the surrogate germline DNA of 109 cases from 93 rare "high-risk" PAC kindreds that were collected through the QPCS and OPCS. These included kindreds with 2 or more PAC-affected family members, as well as PAC cases diagnosed at age 50 years or younger. Notably, the prospective follow-up of these PAC registries, as described in Chapter 2, allowed for the collection of samples from multiple PAC-affected family members in 15 kindreds.

We employed whole exome sequencing since this approach focuses on the ~2% of the genome that is protein-coding, and therefore, most likely to house disease-causing alleles.[403] While NGS allows for the unbiased detection of genetic variants across numerous FPC kindreds, overcoming the limitations of reduced penetrance and locus heterogeneity faced by traditional linkage analyses, the analysis of NGS data is not without its own challenges. In particular, searching among the thousands of genetic variants generated in a single NGS run for the causative mutation(s) is analogous to searching for a "needle in a haystack". To narrow down the vast list of genetic variants identified in our study, we employed a candidate gene, filter-based approach. Since DNA repair genes are widely implicated in gastrointestinal malignancies,[404] including PAC, we hypothesized that there are additional DNA repair PAC susceptibility genes. We therefore focused our search for novel PAC susceptibility genes among 513 putative DNA repair genes, and filtered variants based on the hypotheses that disease-causing alleles would be protein-truncating and would be rare in the general population.

Using this approach, we propose several novel candidate DNA repair PAC susceptibility genes, and provide supporting genetic evidence for several of these genes (i.e., segregation and somatic studies). Given that PAC associated with known DNA repair PAC susceptibility genes (*BRCA1*, *BRCA2* and *PALB2*) has been associated with distinct genomic features, therapeutic sensitivities, and possibly clinical outcomes, we also evaluated whether there is a difference in clinical outcome, using overall survival as a surrogate, between carriers *versus* non-carriers of germline DNA repair gene mutations.

4.2 ABSTRACT

The genetic basis underlying the majority of hereditary pancreatic adenocarcinoma (PC) is

unknown. Since DNA repair genes are widely implicated in gastrointestinal malignancies, including PC, we hypothesized that there are novel DNA repair PC susceptibility genes. As germline DNA repair gene mutations may lead to PC subtypes with selective therapeutic responses, we also hypothesized that there is an overall survival (OS) difference in mutation carriers versus non-carriers. We therefore interrogated the germline exomes of 109 high-risk PC cases for rare protein-truncating variants (PTVs) in 513 putative DNA repair genes. We identified PTVs in 41 novel genes among 36 kindred. Additional genetic evidence for causality was obtained for 17 genes, with *FAN1*, *NEK1* and *RHNO1* emerging as the strongest candidates. An OS difference was observed for carriers versus non-carriers of PTVs with early stage (≤ IIB) disease. This adverse survival trend in carriers with early stage disease was also observed in an independent series of 130 PC cases. We identified candidate DNA repair PC susceptibility genes and suggest that carriers of a germline PTV in a DNA repair gene with early stage disease have worse survival.

4.3 INTRODUCTION

Pancreatic ductal adenocarcinoma (PC) has the worst prognosis of any solid tumor type, which is largely attributable to late diagnosis.[339,345] Since genetic predisposition is thought to underlie 10% of PC, early detection programs for individuals at increased risk may improve clinical outcomes. A role for screening programs is supported by estimates that PC develops over a decade following the initiating somatic mutation, providing significant lead-time for screening.[254] Unfortunately, screening strategies based on family history alone have been largely ineffective.[241,246] An understanding of the full spectrum of causative germline mutations will help identify individuals at highest risk and allow for more specific screening programs.

The evidence for hereditary PC is based on familial clustering suggestive of Mendelian inheritance, as well as the occurrence of PC within the tumor spectrums of characterized genetic syndromes.[224] Family history is an important risk factor for PC, with a 2.3- to 32-fold increased risk depending on the number and relatedness of affected relatives in a family.[223] Hereditary PC occurring either alone or as part of a tumor spectrum in families is partially attributable to rare, loss-of-function mutations, usually protein-truncating variants (PTVs) in the *BRCA2, BRCA1, PALB2, ATM, CDKN2A, PRSS1, SPINK1* and mismatch repair (*MLH2, MSH2, MSH6* and *PMS2*) genes.[82] However, these genes account for less than 10% of hereditary PC, and one of the most important questions in the field remains the identification of the genetic causes where known genes are not implicated.

Segregation analyses suggest autosomal dominant inheritance of a rare allele(s) with variable penetrance to explain the missing heritability of PC.[255] However, traditional linkage and genome-wide association studies have been largely unsuccessful in identifying novel medium or high penetrant PC susceptibility loci.[82,280] This is likely owing to unavailability of DNA from multiple affected family members due to the rapid progression of PC, locus heterogeneity, and variable penetrance of disease-causing alleles.[82] The unbiased nature of next generation sequencing (NGS) overcomes many of these limitations, making this a promising approach for discovery of novel PC susceptibility genes, as evidenced by the recent identification of *PALB2* and *ATM* using this approach.[136,137]

Searching among thousands of genetic variants identified by NGS for the causative mutation is analogous to identifying the proverbial “needle in a haystack”. An *a priori* candidate gene approach is one method of overcoming this challenge and has been successful in identifying novel cancer susceptibility genes.[277,405,406] Since DNA repair genes are widely implicated in

gastrointestinal malignancies,[404] and account for the majority of hereditary PC attributable to known PC predisposition genes (*BRCA1, BRCA2, ATM, PALB2*, mismatch repair genes),[223,224] we hypothesized that additional DNA repair genes are involved in hereditary PC. Therefore, we employed a DNA repair candidate gene approach to interrogate whole exome sequencing (WES) data for novel susceptibility genes. In addition, since there is a growing body of literature suggesting that PC associated with germline mutations in homology-directed DNA repair (HDR) genes (i.e., *BRCA1, BRCA2, PALB2*) have distinct genomic signatures, therapeutic responses and possibly clinical outcomes,[37,311,314] we questioned whether there is an overall survival (OS) difference in carriers versus non-carriers of germline mutations in putative DNA repair genes.

4.4 MATERIALS AND METHODS

4.4.1 Participants

PC cases enrolled in the Ontario[333] or Quebec[364] Pancreas Cancer Studies (OPCS, QPCS) were selected for WES of lymphocyte or white blood cell (surrogate germline) DNA. This series of cases (discovery set) included 8 young onset cases (diagnosed at 50 years of age or less) and 101 cases from 85 families with two or more PC-affected relatives (Table S1). These cases were not known to carry causal mutations in known PC susceptibility genes (i.e., *BRCA2, BRCA1, PALB2, ATM, CDKN2A, PRSS1, SPINK1* and mismatch repair genes). Of the familial cases, WES data were generated from 70 cases for which DNA was available from a single affected family member and in 15 families where DNA was available from multiple PC-affected family members. We also generated WES data from matched tumor DNA that was available for cases 52B and 72. Fresh-frozen tumor samples for these cases were macrodissected to enrich for higher tumor cellularity prior to extracting DNA for WES. For case 58B, we used existing tumor whole genome

sequencing (WGS) data.[407] Available tissues from relatives of patients included in the discovery set were used for segregation and loss of heterozygosity (LOH) studies. These individuals were also enrolled in the OPCS or QPCS.

The validation set was composed of 130 PC cases with existing WGS data.[407] All of these cases were explored surgically for resectability. This independent series of PC cases did not carry predisposing germline mutations in known genes and were unselected for increased hereditary PC risk.

The study was approved by the McGill University and Mount Sinai Hospital ethics review boards and written informed consent was obtained for all participants.

4.4.2 Whole exome sequencing

Library capture, variant calling and filtering (Figure 1) methods are described in Supplemental Materials and Methods.[370-372,393-396,408,409] We evaluated for rare PTVs in genes implicated in DNA repair (n=513; Table S2) and in recognized PC susceptibility genes that are not implicated in DNA repair (i.e., *CDKN2A, PRSS1,* and *SPINK1*). Table S2 lists the 513 recognized and putative DNA repair genes evaluated, which includes genes defined as DNA repair genes in the Gene Ontology project (via AmiGO browser),[410] genes included in the REPAIRtoire database[411] and other genes identified through PUBMED literature search. Primers used to validate variants by Sanger sequencing are listed in Table S3.

4.4.3 Segregation

Segregation of variants with PC was assessed in kindreds where WES data were available from multiple PC-affected family members. In cases where archived formalin-fixed, paraffin-

embedded (FFPE) non-tumor tissue samples were available from relatives affected with PC, genomic DNA was extracted and tested for segregation by Sanger sequencing (see Table S4 for primers). In kindreds where samples were unavailable from PC-affected family members, DNA available from unaffected family members was used to infer segregation.

4.4.4 Loss of heterozygosity

In cases where tumor WES (52B, 72) or WGS[407] (58B) data were available, variants were assessed for LOH or somatic inactivation of the second allele. In cases where archived FFPE tumor blocks were available, LOH was assessed by Sanger sequencing (see Table S4 for primers). Regions of tumor cellularity >50% were macrodissected prior to DNA extraction. LOH was analyzed by visually comparing allelic ratios of tumor and respective normal tissue DNA.

4.4.5 Overall survival

Univariate and multivariate Cox proportional hazard models were used to identify variables impacting survival. OS, defined as the time from diagnosis to death by any cause, was chosen as the primary end-point since this closely reflects cancer-related death in PC. Censoring events were created for patients alive at the time of last follow-up or patients lost to follow-up. Date of diagnosis was defined as date of first documentation of radiologic evidence or biopsy confirmation of PC. Date of surgery was used in cases where date of diagnosis was unknown. We included only the affected probands from kindreds in which multiple PC-affected relatives were sequenced. Covariates included age at diagnosis, gender, stage [early stage (≤ IIB) versus advanced stage (≥ III)] and DNA repair PTV carrier status (carrier versus non-carrier). Chemotherapy status was unavailable for 14 patients and was therefore excluded as a covariate. The results are reported as

hazard ratios (HRs) with 95% confidence intervals (CIs). Kaplan-Meier survival curves were generated for carriers versus non-carriers of DNA repair gene PTVs and compared using the log-rank test. All statistical analyses were performed using SPSS version 22.0 (SPSS Inc., Chicago, IL, USA). Statistical significance was defined as $P \leq 0.05$.

Existing WGS data from an independent series of 130 PC cases[22] were used to validate the OS correlations observed in the discovery set. Variant calling and filtering steps for these data are described in the Supplemental Materials and Methods. The OS analyses were carried out as described for the discovery set, with date of surgery used as a surrogate for date of diagnosis for all cases. Family history status [sporadic (n=122) versus familial (n=18)] was included as a covariate.

4.5 RESULTS

4.5.1 Whole exome sequencing

WES data were generated for 109 high-risk PC cases from 93 families, as well as matched tumor DNA from two patients. The mean read depth obtained for target regions was 61.8±39.8 and the average percentage of Consensus Coding Sequence (CCDS)[412] bases covered by at least 5, 10 and 20 reads were 94.0, 89.4 and 77.5, respectively. As expected, coverage was superior in the newer generation capture kits. The average percentage of CCDS bases covered by at least 5 reads were 92.2, 96.7 and 97.5 for the Illumina TruSeq Exome Enrichment Kit (n=69), Agilent SureSelect Human All Exon V4 (n=14) and Roche NimbleGen SeqCap EZ kit v3.0 (n=26) kits, respectively. The mean read depth obtained for the tumor exomes (n=2) was 130.8±3.3, and the average percentage of CCDS bases covered by at least 5, 10 and 20 reads were 97.1, 96.1 and 94.5, respectively.

4.5.2 Identification of DNA repair gene variants

Variant filtering was applied to the germline WES dataset (n=109) according to the algorithm outlined in Figure 1. Following quality filtering, a total of 52,933 nonsynonymous variants remained. Of these, 2,569 variants were PTVs. Next, variants were excluded if homozgyous or if present at a MAF >0.005 in unaffected in-house control exomes (n=1045), the 1000 Genomes Project or the NHLBI Exome Variant Server, leaving 1,905 rare PTVs. Of these, variants in recognized and putative DNA repair genes (n=513, Table S2) were selected for further evaluation. We also evaluated for PTVs in known PC susceptibility genes that are not implicated in DNA repair (i.e., *CDKN2A, PRSS1,* and *SPINK1*). A total of 70 variants in 56 DNA repair genes were identified. Following visual inspection, 48 variants (68.6%) in 44 genes remained. Sanger sequencing confirmed 45 PTVs in 42 DNA repair genes, resulting in a validation frequency of 93.8%. Of the confirmed PTVs, 16 were nonsense, 20 were frameshift indels, and 9 were splice-site variants (Table S5).

Forty-one PC cases in 37 (39.8%) kindreds had one or more PTVs in a DNA repair gene (Table S5). Notably, we identified one previously unrecognized PTV in a known PC susceptibility gene [*BRCA2*:c.4691dupC (p.Thr1566Aspfs*9)]. Of the remaining 41 novel genes identified in 36 (38.7%) kindreds, four genes (*FANCL, MC1R, NEK1* and *RHNO1*) had PTVs in multiple kindreds. Seven individuals were carriers of two PTVs, one individual was a carrier of 3 PTVs, while 2 kindreds had different affected family members carrying different PTVs (Table S6).

To further prioritize candidate genes, the WES data were also searched for nonsynonymous (missense and in-frame indel) variants in the 41 putative DNA repair genes that were confirmed by Sanger sequencing (Figure 1). The same quality and control filtering were utilized as for PTVs.

Excluding variants annotated as "benign" in ClinVar[370] and considering only missense variants predicted to be pathogenic by 4 *in silico* prediction tools, 18 missense variants and 2 in-frame indels in 16 DNA repair genes were identified (Table S7). All variants were confirmed by Sanger sequencing. Twenty-two PC cases in 19 (20.4%) kindreds had one or more missense variant or in-frame indel (Table S7). Three PC cases were carriers of multiple nonsynonymous variants and five cases were carriers of both a PTV and one or more nonsynonymous variant (Table S6). Five genes (*DCLRE1A, FAN1, POLQ, TEX15, TONSL*) had a missense variant or in-frame indel in multiple kindreds (Table S7).

4.5.3 Segregation and loss of heterozygosity analyses

For all validated variants, segregation with PC was assessed in families where either WES sequencing data were available from affected family members or DNA was available from affected or unaffected relatives (Tables S5 and S7). Fourteen genes demonstrated segregation of variants with PC in two or more affected family members, including *AATF, BLM, CEP164, CHD1L, FAN1, FANCG, MC1R, NEIL1, NEK1, NEK11, RHNO1, SPP1, TONSL,* and *WRN*. Notably, the following variants were found to co-segregate in 3 affected family members: *NEK11*:c.455+1G>A, *SPP1*:c.94-1G>A and *FAN1*:c.149T>G (p.Met50Arg). Five genes had variants segregating in 2 families: *AATF, CHD1L, FAN1, NEK1* and *RHNO1*.

LOH was assessed in all cases where tumor WES data were available or in cases where archived FFPE tumor blocks were available. In total, LOH was assessed for 27 variants in 29 tumors, with loss of the wild-type allele observed for three variants [*MGMT*:c.593G>A (p.Trp198*), *RHNO1*:c.250C>T (p.Arg84*), *WDR48*:c.1278_1279del (p.Gly427Aspfs*8)], heterozygosity retained for 22 variants in 24 tumors and loss of the alternate allele observed for

two variants [*MLH3*:c.1856A>T (p.Lys619Ile) and *PMS1*:c.1826G>A (p.Trp609*)]. Additionally, no second somatic mutation was observed in the tumor WES data for cases 52B and 72 carrying variants *MC1R*:c.456C>A (p.Tyr152*) and *NINL*:c.4142_4143del (p.Ser1381Cysfs*17), respectively, or in the tumor WGS data for case 58B carrying the *FAN1*:c.149T>G (p.Met50Arg) variant.

4.5.4 Top candidate genes

Among the 41 putative DNA repair genes identified with at least 1 PTV among high-risk PC cases, 17 genes have stronger genetic evidence supporting their roles as candidate novel PC predisposition genes (Table 1). This includes genes with more than 1 kindred with a PTV in that gene, genes with segregation of a predicted-pathogenic variant in at least one kindred and/or genes with at least one predicted-pathogenic variant and LOH of the corresponding wild-type allele. Of particular note are *FAN1*, *NEK1* and *RHNO1,* which have variants present in 3 kindred and co-segregation of a variant with PC in at least 2 kindred. Figures 2, 3 and 4 show the pedigrees for families carrying variants in *FAN1*, *NEK1* and *RHNO1*, respectively.

4.5.5 Overall survival - Discovery set

The results of the univariate and multivariate Cox models of variables implicated in OS in the discovery set are shown in Table S8. Seventy-five (82.4%) cases were deceased. Considering all stages combined (n=91), significant associations were found for stage (early versus advanced) in both univariate and multivariate analyses (HR 4.3, 95% CI 2.5-7.0; $P<0.001$ and HR 5.9, 95% CI 3.4-10.4, $P<0.001$, respectively), and age at diagnosis in multivariate analysis (HR 1.03, 95% CI 1.01-1.05; $P=0.003$). Subset analyses were carried out for patients who presented with early (≤

IIB, n=50) and advanced (≥ III, n=41) stages. Interestingly, carriers of DNA repair gene PTVs with early stage (≤ IIB) had worse OS by univariate and multivariate analyses (HR 2.5, 95% CI 1.2-5.0; *P*=0.010 and HR 2.4, 95% CI 1.2-4.9, *P*=0.015, respectively). However, for patients with advanced stage (≥ III), carrier status did not correlate with OS. Figure 5 (panels A to C) show the Kaplan-Meier survival curves for carriers of DNA repair gene PTVs versus non-carriers.

4.5.6 Overall survival - Validation set

The WGS data of the 130 PC cases in the validation set were assessed for rare germline PTVs in the 41 candidate DNA repair genes identified in the discovery set. We found 10 PTVs in 8 genes [*AATF*, *BLM*, *CHD1L*, *DCLRE1A* (2), *NEK1*, *POLL, POLQ*, *TEX15* (2)] (Table S9). Ninety-nine (76.2%) cases were deceased. The results of the univariate and multivariate Cox regression analyses are shown in Table S10. Considering all stages combined (n=130), stage was a significant factor by univariate and multivariate analyses (HR 3.58, 95% CI 1.7-7.5; *P*=0.001 and HR 4.2, 95% CI 1.9-9.1, *P*<0.001, respectively), and a significant survival disadvantage for carriers of DNA repair gene PTVs versus non-carriers was observed by multivariate analysis (HR 2.8, 95% CI 1.2-6.3; *P*=0.017). Considering early stage alone (n=122), carrier status remained a significant variable in both univariate and multivariate analyses (HR 2.6, 95% CI 1.3-5.5; *P*=0.011 and HR 3.1, 95% CI 1.4-6.7; *P*=0.006 in univariate and multivariate analyses, respectively). The validation case series was enriched for early stage cases, with only 8 patients presenting with advanced stage disease and none carrying PTVs. The Kaplan-Meier survival curves for carriers of DNA repair gene PTVs versus non-carriers for all stages and early stage, respectively, are shown in Figure 6 (panels A and B).

We also evaluated the 130 cases in the validation set for rare PTVs in all 513 putative DNA repair genes (Table S2) and repeated the OS analysis. We observed 39 PTVs in 34 genes in 33 cases (25.4%; Table S9). The results of the Cox regression analyses are shown in Table S11. For all stages combined (n=130), stage remained a significant factor (HR 3.8, 95% CI 1.8-8.3, *P*=0.001 in multivariate analysis). Considering all stages, a survival disadvantage for carriers of DNA repair gene PTVs versus non-carriers was observed in both univariate and multivariate analyses (HR 1.7, 95% CI 1.1-2.7; *P*=0.024 and HR 1.7, 95% CI 1.1-2.7, *P*=0.022, respectively). For early stage alone (n=122), carrier status retained association with worse OS in univariate and multivariate analyses (HR 1.6, 95% CI 1.0-2.7; *P*=0.046 and HR 1.6, 95% CI 1.0-2.7; *P*=0.051, respectively). The Kaplan-Meier survival curves for carriers of DNA repair gene PTVs versus non-carriers for all stages, early stage and advanced stage are shown in Figure 6 (panels C to E).

4.6 DISCUSSION

We report a large-scale NGS study aimed at identifying novel genetic causes of hereditary PC in which 109 high-risk PC cases from 93 families underwent WES. Using a filter-based candidate gene approach focused on DNA repair genes, we identified PTVs in 41 putative DNA repair genes among 36 (38.7%) kindreds. We also found a rare PTV in *BRCA2*, a known PC susceptibility gene, demonstrating the ability of the approach to identify causal variants. The WES data were also evaluated for mutations in known PC predisposition genes not implicated in DNA repair (i.e., *CDKN2A, SPINK1* and *PRSS1*). Since WES is unable to detect large genomic deletions and rearrangements, the possibility of such variants in known and candidate PC predisposition genes cannot be excluded and represents a limitation of our study. The 41 candidate genes were

further characterized for predicted pathogenic nonsynonymous variants, segregation of putative pathogenic variants with disease in families, and LOH of the wild-type allele in tumors.

Some of the challenges in identifying causal genes in hereditary PC are the occurrence of phenocopies, genetic heterogeneity, and variable penetrance of disease-causing alleles.[82] Although young age of onset is a risk factor for hereditary cancer, the majority of hereditary PC cases have the same age of onset as sporadic cases.[233] As such, genetic studies of hereditary PC are often confounded by phenocopies. This notion is highlighted by our previous report showing lack of segregation of the *PALB2:c.3256C>T (p.Arg1086*)* and *ATM*:c.1931C>A (p.Ser644*) PTVs with PC-affected relatives.[86] Consequently, in the present study, we used segregation status of variants to prioritize but not exclude candidate genes.

Although LOH was viewed favourably for causation, its absence did not exclude candidate genes since there are other mechanisms of somatic loss of the wild-type allele, as well as the possibility that haploinsufficiency is sufficient for tumorigenesis. Consistent with this possibility, the *BRCA2*:c.4691dupC (p.Thr1566Aspfs*9) variant identified in the present study did not exhibit LOH, suggesting other mechanisms of wild-type allele silencing in the tumor.

Another challenge in identifying causative genes in hereditary PC is the presence of multiple predicted-pathogenic variants in a single individual. As shown in Table S6, we observed cases with PTVs or predicted-pathogenic missense variants in multiple putative DNA repair genes. Double heterozygosity of pathogenic variants in multiple cancer predisposition genes has been previously reported in breast and ovarian cancers and likely reflects the variable penetrance of disease-causing alleles, where only one germline variant is needed to drive tumorigenesis.[413,414] The variable penetrance of PC predisposition genes identified to date suggests that the presence of double heterozygotes of disease-causing variants in our high-risk case series is plausible. In

addition, the possibility that mutations in two genes results in predisposition "synergy" in the form of "additive" haploinsufficiency is an interesting hypothesis.

Based on the evidence obtained from the genetic investigations, we prioritized our list of 41 candidate PC susceptibility genes. Top candidate genes (n=17, Table 1) were considered those with more than 1 kindred with a PTV in that gene, genes with segregation of a predicted-pathogenic variant (PTV or nonsynonymous variant) in at least one kindred, and/or genes with LOH associated with at least one predicted-pathogenic variant. The strongest candidate PC predisposition genes, in view of case frequency, segregation and somatic silencing, are *FAN1*, *NEK1* and *RHNO1*. Each of these genes had variants present in 3 out of 94 high-risk kindreds (3.2%), with at least partial co-segregation of the variants with PC in two or more kindreds (Figures 2, 3 and 4).

FAN1 (FANCD2/FANCI-associated nuclease 1) is required for the repair of interstrand cross-links.[415,416] Interestingly, *FAN1* has recently been reported as a putative colon cancer susceptibility gene.[417] Here, we identified a PTV in *FAN1* (p.Arg710*) in Family 42, as well as a missense variant (p.Met50Arg) present in 2 kindreds. While there were no samples available for segregation or LOH analyses of the p.Arg710* variant, the p.Met50Arg variant demonstrated complete co-segregation with PC in tested family members (Figure 2). Notably, the p.Met50Arg variant occurs at a highly conserved amino acid residue within the RAD18-like ubiquitin-binding (UBZ) domain, which is essential for FAN1 localization to sites of DNA damage.[415] The absence of LOH in two tumors from carriers of the p.Met50Arg variant and lack of evidence of a second hit in one of these cases (58B) for which WGS was available, is consistent with the results reported by Seguí et al,[417] where somatic inactivation of the wild-type allele was not observed in any of the colon cancer cases with germline *FAN1* mutations.

NEK1 [NIMA (Never In Mitosis Gene A)-Related Kinase 1] is the second strongest candidate gene. Its protein product is a dual serine-threonine and tyrosine kinase required for efficient DNA damage checkpoint activation and for maintaining chromosome stability.[418] Moreover, there is evidence to suggest that NEK1 functions as a tumor suppressor.[418] In the present study, 2 of 93 high-risk PC families were found to carry a novel *NEK1*:p.Ala563Tyrfs*36 variant. This variant has not been previously reported in the public control databases,[372,409,419] nor was observed in 1,045 in-house control exomes. One family is of Greek origin and the other of English and Scottish descent. Therefore, this recurrent variant is unlikely to represent cryptic relatedness or an ethnic-specific variant but, perhaps, a mutation "hot spot" in *NEK1* (deletion occurs within a triple "AG" repeat). Segregation of this variant was observed in one bilineal family (78), and only partial segregation was observed in the second family (17) (Figure 3). Notably, the affected relative found to be wild-type for the *NEK1* variant in Family 17 was the eldest diagnosed in the family (75 years of age) and may represent a phenocopy. A third family was found to carry the predicted-pathogenic p.Asn648Lys variant with segregation of the variant observed in the 2 PC-affected siblings. As well, an additional PTV in *NEK1* (c.868+1G>C) was identified in the case series used for the OS validation studies, providing further support for *NEK1* as a candidate PC susceptibility gene.

Interestingly, *NEK1* maps to chromosomal region 4q33, within a previously reported PC susceptibility locus (4q32-34) identified by linkage analysis of a kindred with 9 PC-affected family members and additional relatives with precancerous pancreatic lesions.[235] Sequencing of candidate genes in the region in affected family members led to the identification of a variant in *PALLD* (P239S) which is considered to be the causative mutation in this family.[264] Although *PALLD* may be causative in this kindred, subsequent studies have not supported *PALLD* as a common PC

susceptibility gene.[268-270] Interestingly, *NEK1* was among the candidate genes sequenced in the aforementioned study and even though sequencing failed to identify a *NEK1* variant, assays for large genomic structural changes at this locus were not performed. Thus, the possibility that *NEK1* underlies PC predisposition in this kindred cannot be fully excluded.

The third top ranking candidate gene is *RHNO1* (Rad9-Hus1-Rad1 Interacting Nuclear Orphan 1), which has an important role in DNA damage response signaling.[420] Two PC kindreds were found to carry different PTVs in *RHNO1*. The p.Arg84* variant was not found to segregate with PC in the second affected family member tested (Figure 4), but LOH of the wild-type allele was observed in the proband tumor. The p.Arg113* variant was found to segregate with both PC-affected family members. A third family was found to carry the p.Leu16Val predicted-pathogenic missense variant, with co-segregation of the variant among PC-affected third degree relatives. This variant has also been observed in a thyroid cancer sample reported in the COSMIC database (COSM4146987). It is noteworthy that a recent study did not observe a statistically significant difference in the frequency of inactivating *RHNO1* mutations (including the p.Arg84* and p.Leu16Val variants identified in the present study) among Finnish breast cancer families versus population controls.[421] However, this finding does not exclude a possible role for *RHNO1* in PC predisposition.

Additional genes identified in the present study have been implicated in other hereditary cancer syndromes and might also have a role in PC susceptibility. In particular, *BLM* has been implicated in hereditary breast cancer and *BARD1* has been implicated in hereditary breast and ovarian cancers.[405,422] The Fanconi Anemia genes, *FANCG* and *FANCL*, are also noteworthy since HDR genes are of particular interest in PC.[18-21] A nonsynonymous variant previously associated with Fanconi Anemia in *FANCG* has been previously described in a cell line derived from an early

onset PC and demonstrated LOH.[273] However only intronic variants were observed in a follow up study of 38 familial PC kindreds.[274] Another gene of interest is *POLQ,* which has recently been shown to have a key role in the microhomology-mediated end joining of double stranded DNA breaks, and has been suggested as a potential target for synthetic lethality in HDR-deficient tumors.[423,424] One PTV and three predicted-pathogenic missense variants were identified in five cases from the discovery set, however the variants did not segregate with PC in the four families that were tested. An additional PTV was identified in the validation set.

Since PC is likely a heterogeneous disease,[37] it is possible that causative germline mutations among genes with similar cellular roles (i.e., DNA repair) may give rise to unique PC clinical subtypes. Therefore, we evaluated whether carriers versus non-carriers of PTV germline variants in a putative DNA repair gene have different clinical outcomes. We used OS as a marker of clinical outcome since these clinical data were available and OS closely reflects cancer deaths due to the lethality of PC. Only PTVs were considered in these analyses since missense variants were primarily evaluated as a means of prioritizing candidate genes in the discovery set as pathogenicity cannot be concluded with the same degree of confidence in the absence of functional assays, despite our strict *in silico* selection criteria. In the discovery set, we found a significant adverse difference in OS among carriers versus non-carriers of DNA repair gene PTVs in cases with early (≤ IIB), but not advanced (≥ III), stage disease.

This adverse OS for carriers versus non-carriers was validated in an independent series of 130 PC cases.[407] We first confirmed a survival disadvantage for carriers of PTVs of the 41 genes identified in the discovery set. Next, we searched for PTVs in the full panel of 513 putative DNA repair genes, since additional genes not identified in the discovery set may be contributory. The adverse OS trend with carrier status persisted when we examined all 513 genes. The carrier status

association with survival that was observed for all stages in the validation, but not in the discovery, set likely reflects the sampling bias of the validation set (only 8 patients with advanced stage). Most notably, a survival disadvantage was observed in both the discovery and validation sets for early, but not advanced, stage disease. Since early stage patients are expected to have better outcomes, the adverse survival observation for these cases is intriguing and cannot be explained by an earlier stage selection bias.

Although the OS correlation with carrier status needs to be validated in a larger case series of prospectively collected cases, our observations points to a hypothesis that PC patients with inherent DNA repair deficiencies, perhaps even haploinsufficient, may have a distinct clinical outcome. This concept is not without precedent. PC tumors from patients with germline mutations in *BRCA1*, *BRCA2* and *PALB2* show unique treatment responses to DNA-damaging agents (e.g., platinums and PARP inhibitors).[37,311,314] As well, Waddell et al.[37] have recently described an "unstable" genomic subtype of PC defined by a large number of structural variation events that reflect underlying defects in DNA maintenance. This unstable PC subtype was associated with inactivation of DNA repair genes (*BRCA1*, *BRCA2* and *PALB2*). Interestingly, only half of tumors within this group were accounted for by germline or somatic mutations in these three genes, suggesting that additional DNA repair genes may be important. Our findings that PTV carrier status is associated with an adverse clinical outcome may reflect a more aggressive PC subtype and/or a PC subtype with unique therapeutic sensitivities, akin to mismatch repair gene mutation carriers in colon cancer.[425] Since gemcitabine was predominantly used to treat both early and advanced PC stages in the era in which these cases were collected, our observations may be reflecting poor efficacy, or perhaps even a deleterious effect, of gemcitabine in cases with germline DNA repair mutations.

In summary, we have undertaken the first detailed characterization of germline variants in putative DNA repair genes, using NGS, in a large series of selected cases with increased risk of genetic PC predisposition. Our findings suggest that several novel DNA repair genes may have a role in hereditary PC. The heterogeneity of PC susceptibility (i.e., 12 susceptibility genes described to date) and the failure of previous linkage studies to identify major causal loci, suggest that the remaining familial aggregation of PC may be due to several genes, with each gene accounting for only a small fraction of PC susceptibility. Although our study does not provide confirmatory evidence for the candidate genes described, we have prioritized these genes based on available genetic data and propose *FAN1*, *NEK1* and *RHNO1* as the strongest candidates, providing an opportunity for further validation using additional kindreds with PC. The observed survival trend suggests that patients with mutations in DNA repair genes may have more aggressive disease or tumor subtype(s) requiring tailored treatment approaches and warrants validation in a larger series of prospectively collected cases. Such advances will help with the molecular cataloguing of PC as well as the development of gene-based early detection strategies and targeted therapies.

4.7 ACKNOWLEDGEMENTS

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4.8 FIGURES

Figure 4.1. Schematic of the exome sequencing data analysis. Variants remaining after each filtering step are indicated. SNV, single nucleotide variant; indel, insertion/deletion; PTV, protein-truncating variant; MAF, minor allele frequency.

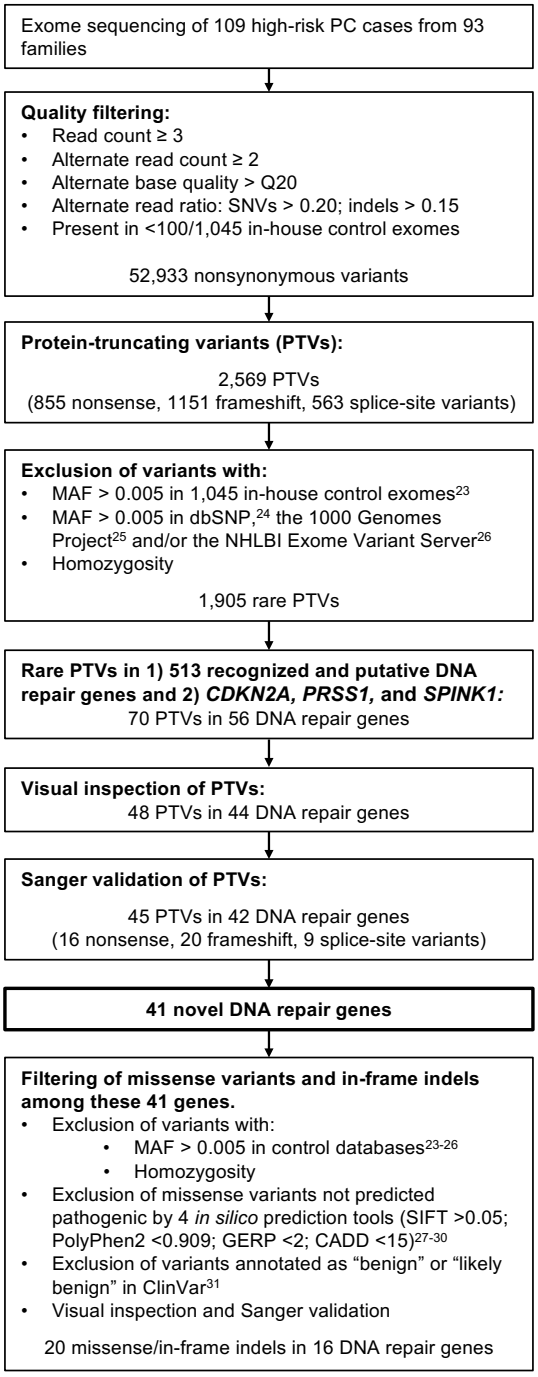


Figure 4.2. Pedigrees of the families with *FAN1* variants. Carrier status is depicted for all the cases in which germline DNA was available and tested. +/- indicates heterozygous carrier status. +/+ indicates wild-type. Probands are indicated with an arrow. Individuals shaded in black are affected with PC, while individuals shaded in grey are affected with a tumor other than PC. The ages of living family members and the ages of death (d.) for deceased individuals are indicated in years. Tumor types and ages at diagnoses are indicated in years. Other illnesses with ages in years at diagnosis (if known) are shown. NHL, non-Hodgkin's lymphoma; CLL, Chronic lymphocytic leukemia.

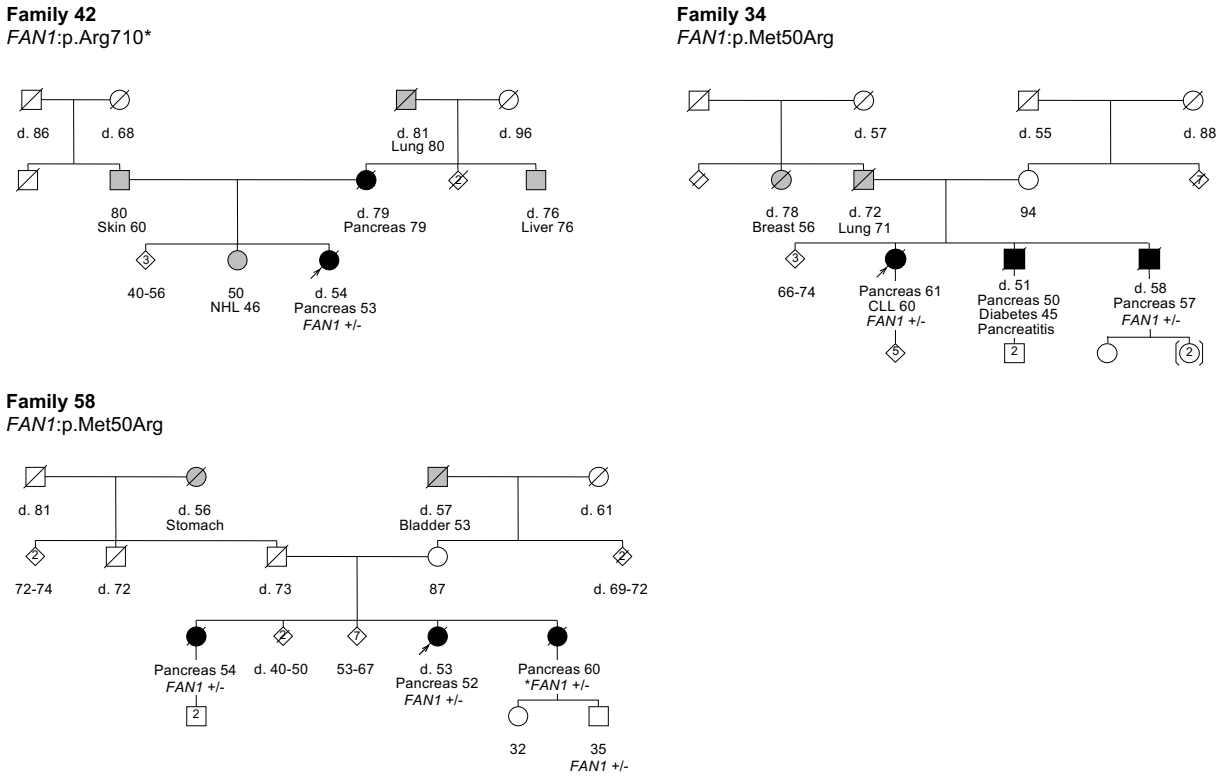


Figure 4.3. Pedigrees of the families with *NEK1* variants. Carrier status is depicted for all the cases in which germline DNA was available and tested. +/- indicates heterozygous carrier status. +/+ indicates wild-type. Probands are indicated with an arrow. Individuals shaded in black are affected with PC, while individuals shaded in grey are affected with a tumor other than PC. The ages of living family members and the ages of death (d.) for deceased individuals are indicated in years. Tumor types and ages at diagnoses are indicated in years. Other illnesses with ages in years at diagnosis (if known) are shown. BCC, basal cell carcinoma.

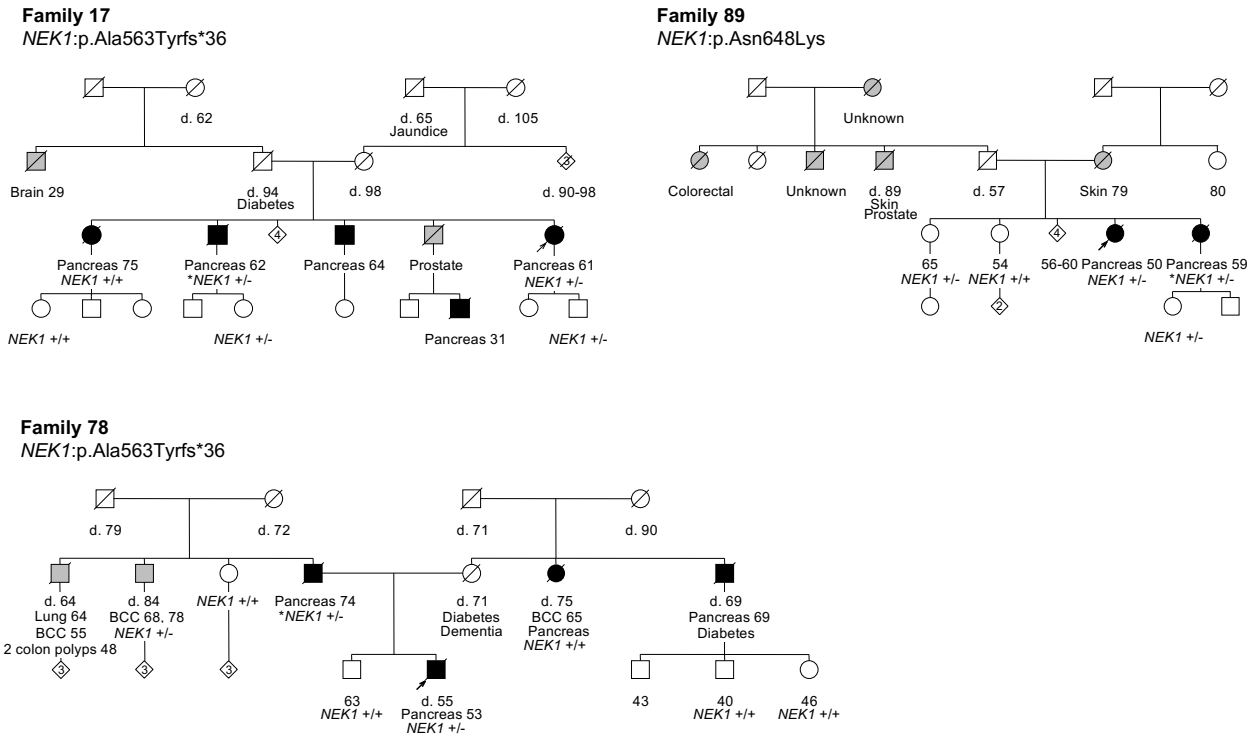


Figure 4.4. Pedigrees of the families with *RHNO1* variants. Carrier status is depicted for all the cases in which germline DNA was available and tested. +/- indicates heterozygous carrier status. +/+ indicates wild-type. Probands are indicated with an arrow. Individuals shaded in black are affected with PC, while individuals shaded in grey are affected with a tumor other than PC. The ages of living family members and the ages of death (d.) for deceased individuals are indicated in years. Tumor types and ages at diagnoses are indicated in years. NM, non-melanoma.

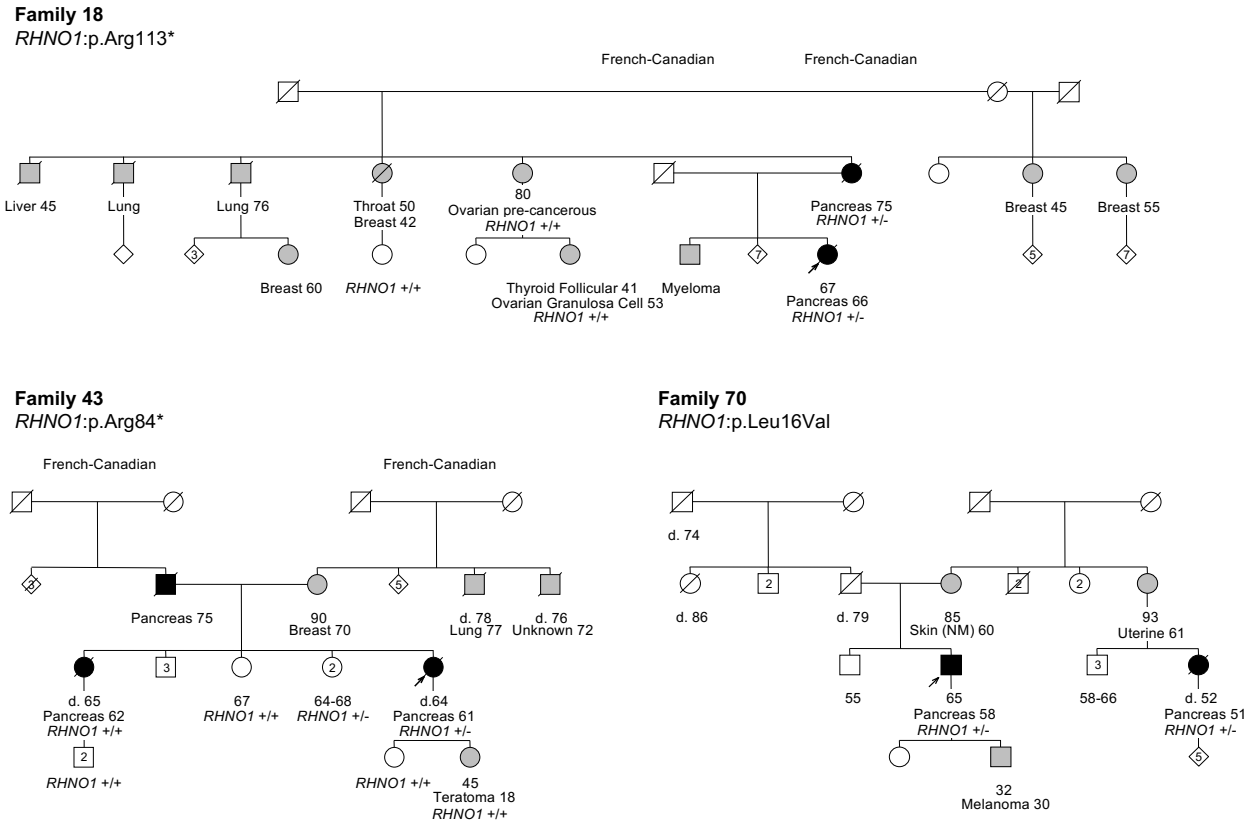


Figure 4.5. Discovery set Kaplan-Meier survival curves for carriers versus non-carriers of DNA repair gene PTVs for all stages (A), early stage (B) and advanced stage (C) cases. Log-rank p-values are indicated.

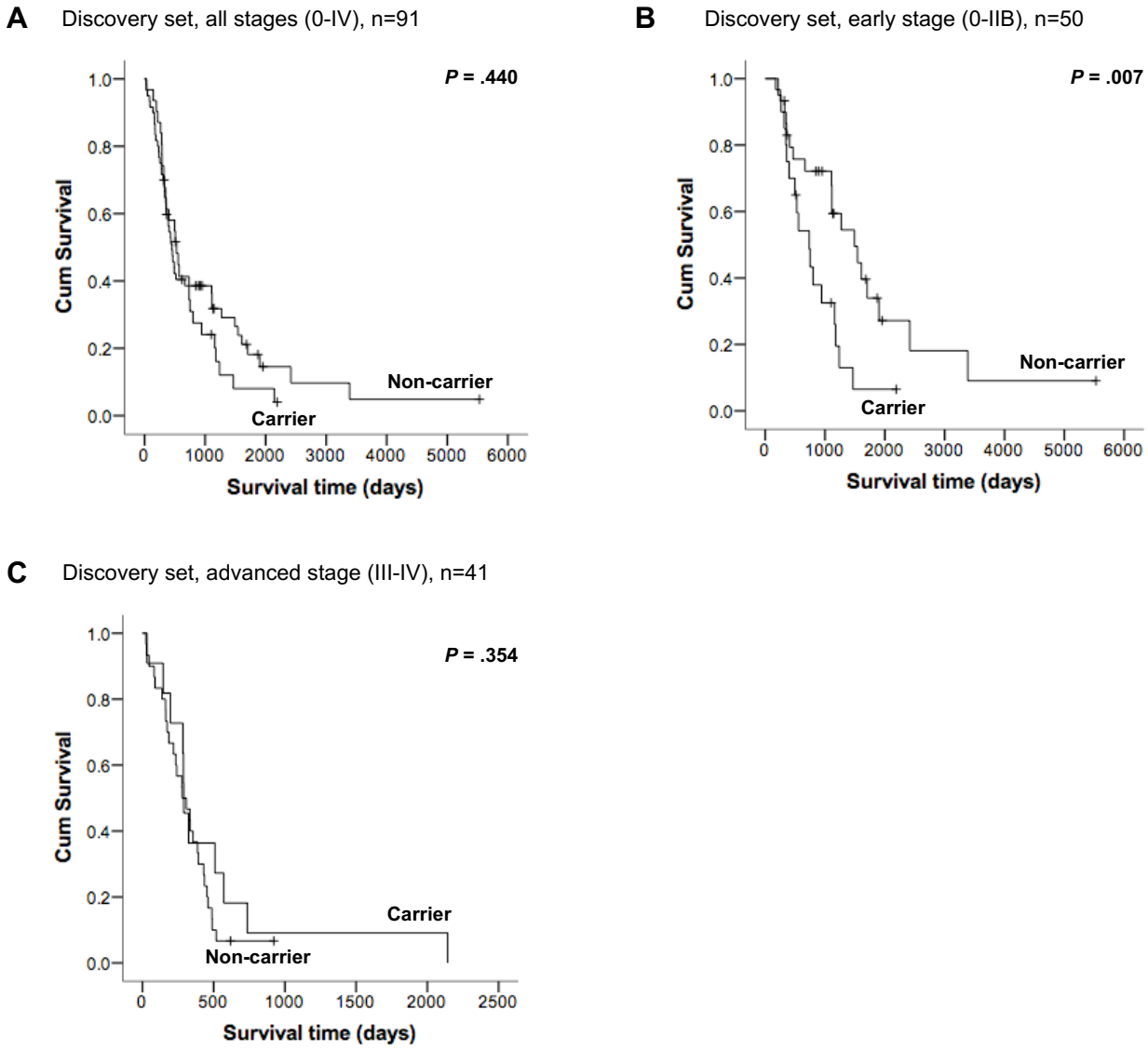
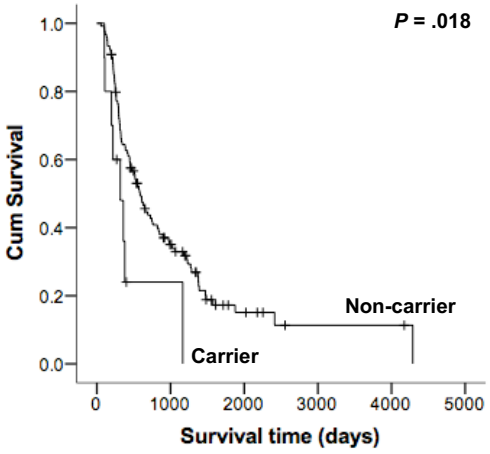
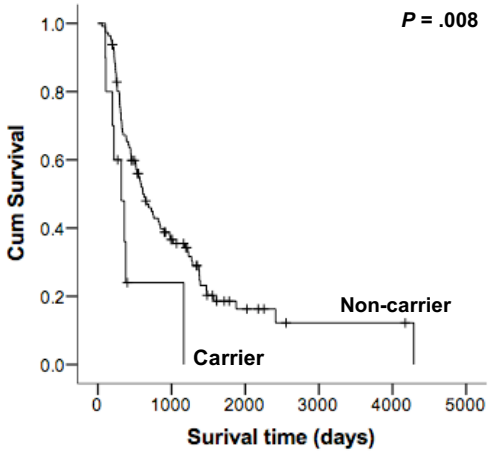


Figure 4.6. Validation set Kaplan-Meier survival curves for carriers versus non-carriers of PTVs in the genes identified in the discovery set (n=41) for all stages (A) and early stage (B), as well as for all 513 putative DNA repair genes (n=513) for all stages (C), early stage (D) and advanced stage (E) cases. Log-rank p-values are indicated.

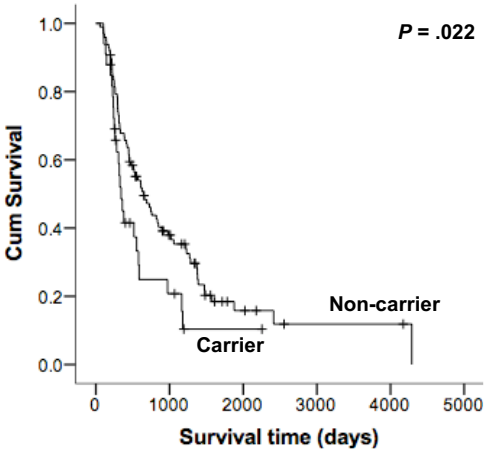
A Validation set, all stages (0-IV), n=130
Genes identified in discovery set, n=41



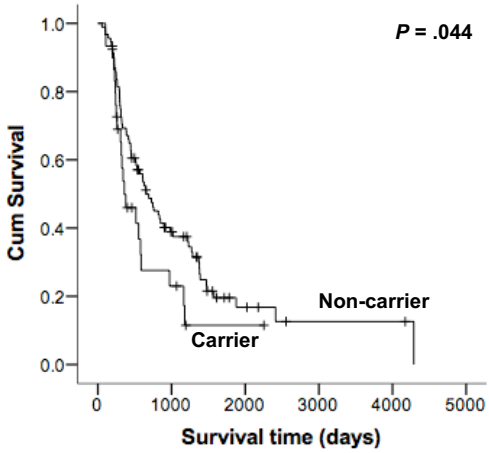
B Validation set, early stage (0-IIB), n=122
Genes identified in discovery set (n=41)



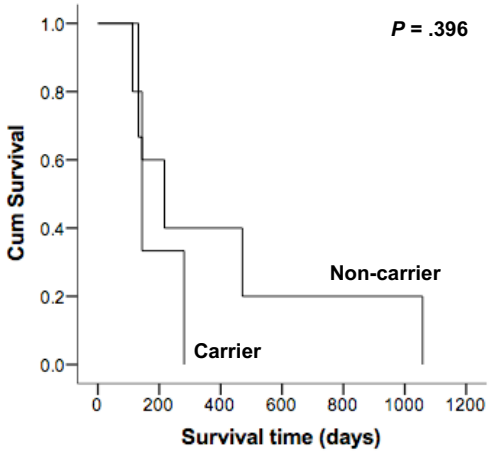
C Validation set, all stages (0-IV), n=130
All putative DNA repair genes, n=513



D Validation set, early stage (0-IIB), n=122
All putative DNA repair genes, n=513



E Validation set, advanced stage (III-IV), n=8
All putative DNA repair genes, n=513



4.9 TABLES

Table 4.1. Top candidate PC susceptibility genes.

Gene	Chr. Pos.	Variant (HGVS nomenclature)	Samples	> 1 PTV	Segregation	LOH
AATF	chr17:35307578	c.158_159dup (p.Gly54Trpfs*157)	76A, 76B		Y	
AATF	chr17:35311130	c.755A>G (p.Asn252Ser)	32		Y	
BLM	chr15:91292792	c.298_299del (p.Gln100Glufs*42)	78B		Y	
CEP164	chr11:117282575	c.4228C>T (p.Gln1410*)	16			
CEP164	chr11:117244534	c.1220C>T (p.Ser407Phe)	53A, 53B		Y	
CHD1L	chr1:146742591	c.1086-2A>G	90		Y	
CHD1L	chr1:146756048	c.1730G>A (p.Gly373Asp)	25		Y	
FAN1	chr15:31214513	c.2128C>T (p.Arg710*)	42			
FAN1	chr15:31197015	c.149T>G (p.Met50Arg)	58A, 58B, 34		Yx2	
FANCG	chr9:35074472	c.1652_1655del (p.Tyr551Phefs*7)	50		Y	
FANCL	chr2:58386928	c.1096_1099dup (p.Thr367Asnfs*13)	47, 55B	Y		
MC1R	chr16:89985733	c.67C>T (p.Gln23*)	14	Y		
MC1R	chr16:89985750	c.86dup (p.Asn29Lysfs*14)	69	Y		
MC1R	chr16:89986122	c.456C>A (p.Tyr152*)	52B	Y	Y	
MC1R	chr16:89986522	c.862_864del (p.Ile288del)	89	Y		
MGMT	chr10:131565137	c.593G>A (p.Trp198*)	63A			Y
NEIL1	chr15:75641315	c.330_331insAGGC (p.Ala111Argfs*46)	43		Y	
NEK1	chr4:170428209	c.1687_1688del (p.Ala563Tyrfs*36)	17, 78	Y	Y	
NEK1	chr4:170398474	c.2235T>G (p.Asn648Lys)	89	Y	Y	
NEK11	chr3:130828766	c.455+1G>A	68C, 68B		Y	
RHNO1	chr12:2997158	c.250C>T (p.Arg84*)	43	Y		Y
RHNO1	chr12:2997245	c.337C>T (p.Arg113*)	18	Y	Y	
RHNO1	chr12:2994578	c.45_46delinsAG (p.Leu16Val)	70A, 70B	Y	Y	
SPP1	chr4:88901197	c.94-1G>A	78A, 78B		Y	
TONSL	chr8:145668147	c.490del (p.Leu164Serfs*72)	2			
TONSL	chr8:145660507	c.2899C>T (p.Arg967Cys)	3A			
TONSL	chr8:145662005	c.1950C>G (p.Asp650Glu)	86		Y	
WDR48	chr3:39125749	c.1278_1279del (p.Gly427Aspfs*8)	72			Y
WRN	chr8:30999118	c.3138+2T>G	51			
WRN	chr8:31012237	c.3785C>G (p.Thr1262Arg)	44		Y	

Chr. Pos., chromosomal position; >1 PTV, more than one kindred with a PTV in the corresponding gene; segregation, segregation of the variant in 2 or more PC-affected family members within a kindred; LOH, loss of heterozygosity (loss of the wild-type allele); Y, yes; x2, occurs in 2 kindreds.

4.10 SUPPLEMENTAL MATERIALS & METHODS

Whole exome sequencing

Library capture varied among samples due to the rapid evolution of sequencing technologies during the course of patient enrolment. Exome capture was completed according to manufacturers' protocols using the Illumina TruSeq Exome Enrichment Kit (Illumina Inc., San Diego, CA, USA; n=69), Agilent SureSelect Human All Exon V4 (Agilent, Santa Clara, CA, USA; n=14) or Roche NimbleGen SeqCap EZ kit v3.0 (Roche NimbleGen Inc., Madison, WI, USA; n=26). Tumor DNA was prepared using the Illumina TruSeq Exome Enrichment Kit (n=2). All post-enrichment DNA libraries were sequenced on Illumina HiSeq2000 platforms with 100 base paired-end reads (Illumina Inc., San Diego, CA, USA). Libraries prepared using the Roche NimbleGen SeqCap EZ kit v3.0 were run 4 samples per lane, while all other libraries were run 3 samples per lane.

Whole exome sequencing variant calling (discovery set)

To generate variant calls, FASTQ files were pooled and run in parallel through the following data workflow. Reads were aligned to the human reference genome (UCSC hg19) using the Burrows-Wheeler Alignment tool (BWA 0.5.9).[426] Local realignment around suspected insertions/deletions (indels) was performed using Genome Analysis Tool Kit (GATK) and PCR duplicates were marked using Picard.[427] Single nucleotide variants (SNVs) and indels were identified using the SAMtools mpileup software and variants were annotated using ANNOVAR and custom in-house scripts.[428,429] Variants were annotated for frequency in dbSNP,[371] 1000 Genomes Project,[409] NHLBI Exome Variant Server,[372] Exome Aggregation Consortium

(ExAC),[419] COSMIC[379] and ClinVar,[370] as well as for *in silico* pathogenicity prediction scores SIFT,[393] PolyPhen 2,[394] GERP[395] and CADD.[396]

Identification of protein-truncating variants and non-synonymous variants (discovery set)

Variant calls were first filtered for quality as follows: (i) base quality score ≥Q20, (ii) depth ≥3, with at least 2 alternate reads, and (iii) alternate allele fraction >0.2 for SNVs or >0.15 for indels. Next, protein-truncating variants (PTVs), which include nonsense, frameshift indels and canonical splice-site variants, were extracted since these are most likely to affect protein function. Since PTVs in causal genes are predicted to be rare events, in-house (1,045 exomes from unaffected individuals run through the same data pipeline)[408] and public control databases (dbSNP, 1000 Genomes Project, NHLBI Exome Variant Server)[371,372,409] were used to filter out variants with a minor allele frequency (MAF) >0.005. Variants with homozygous carriers in our case series, in-house control exomes or the NHLBI Exome Variant Server were also excluded since we hypothesized that causal genes follow an autosomal dominant inheritance pattern. Next, we extracted rare PTVs in genes implicated in DNA repair (n=513; Table S2) and in recognized PC susceptibility genes that are not implicated in DNA repair (i.e., *CDKN2A, PRSS1* and *SPINK1*). The 513 DNA repair gene list included genes defined as DNA repair genes in the Gene Ontology project (via AmiGO browser)[410], genes included in the REPAIRtoire database[411] and other genes identified through PUBMED literature search. The resulting rare PTVs were visually inspected using the Integrative Genomics Viewer (IGV) to eliminate possible sequencing artifacts that were not excluded using the quality filters described above.[401] Variants were validated using Sanger sequencing. Primers were designed using the Primer3 software (http://primer3.ut.ee; Table S3).

WES data were searched for nonsynonymous (missense and in-frame indels) variants in all DNA repair genes with at least one Sanger-confirmed PTV (Figure 1). The same quality and control (MAF <0.005) filtering criteria were utilized as for PTVs. Only missense variants at highly conserved residues predicted to be pathogenic by 4 *in silico* prediction algorithms were considered: SIFT (score <0.05)[393], PolyPhen 2 (score >0.909)[394], GERP (score >2)[395] and CADD (c score >15)[396]. Variants annotated in ClinVar[370] as "benign" or "likely benign" were also excluded. Variants were visually inspected and confirmed by Sanger sequencing as described above (see Table S3 for primer details).

Whole genome sequencing variant calling and filtering (validation set)

WGS data were aligned to the GRCh37.p0 reference with BWA[24] (version 0.6.2). The data were merged, collapsed and duplicates were marked using Picard (version 1.90). Variant calling was performed using the Genome Analysis Took Kit (GATK)[427] (version 1.3.16). The data were locally realigned using the IndelRealigner module of GATK. SNV and indel variants were then identified by the UnifiedGenotyper, and filtered using the Variant Filtration module. Only variants with a quality score >Q50 were retained. Variants were then annotated using ANNOVAR (v.2014.07.15). PTVs in DNA repair genes with MAF < 0.005 in the 1000 Genomes Project[409] and the NHLBI Exome Variant Server[372] were considered in the OS analyses. All variants were confirmed by visual inspection in IGV.[401]

4.11 SUPPLEMENTAL TABLES

Table S4.1. Clinical characteristics of the 109 PC cases from 93 kindreds at high-risk for hereditary PC that underwent whole exome sequencing.

Characteristics	
Age at diagnosis, mean±SD (range)	61.3±13.2 (20-93)
Gender, n (%)	
Male	54 (49.5)
Female	55 (50.5)
# PC affected per kindred (n=93), n (%)	
1 (young onset)	8 (8.6)
2	51 (54.8)
3	22 (23.7)
≥ 4	12 (12.9)
PancPRO score*, mean±SD (range)	0.678±0.316 (0.000-0.989)
Stage, n (%)	
0	1 (0.9)
IA	1 (0.9)
IB	3 (2.8)
IIA	15 (13.8)
IIB	36 (33.0)
III	14 (12.8)
IV	38 (34.9)
Unknown	1 (0.9)
Resected, n (%)	
Y	50 (45.9)
N	58 (53.2)
Unknown	1 (0.9)
Chemotherapy, n (%)	
Y	73 (67.0)
N	15 (13.8)
Unknown	21 (19.3)
*Only PancPRO scores of FPC cases considered, n=101	

Table S4.2. 513 putative DNA repair genes.

AATF, ABL1, ACTR5, AKT1, ALKBH1, ALKBH2, ALKBH3, AP5S1, AP5Z1, APEX1, APEX2, APITD1, APLF, APTX, ASCC3, ASF1A, ASTE1, ATF2, ATM, ATMIN, ATR, ATRIP, ATRX, ATXN3, AXIN2, BABAM1, BAP1, BARD1, BAX, BAZ1B, BCCIP, BLM, BRAP, BRCA1, BRCA2, BRCC3, BRE, BRIP1, BTG2, BUB1, BUB1B, C11orf30, C17orf70, C19orf40, CASP3, CCNA1, CCNA2, CCNB1, CCND1, CCNE1, CCNH, CCNO, CDC14B, CDC25A, CDC25B, CDC25C, CDC45, CDC6, CDH13, CDK1, CDK2, CDK4, CDK7, CDKN1A, CDKN1B, CDKN2A, CDKN2D, CEBPG, CEP164, CEP170, CETN2, CHAF1A, CHAF1B, CHD1L, CHD4, CHEK1, CHEK2, CHRNA4, CIB1, CINP, CLSPN, COPS5, CRB2, CREB1, CREBBP, CRY1, CRY2, CSNK1D, CSNK1E, CUL4A, CUL4B, CYP19A1, CYP1A1, DAPK1, DBF4, DCLRE1A, DCLRE1B, DCLRE1C, DDB1, DDB2, DDR1, DDX1, DEK, DHX9, DMAP1, DMC1, DNA2, DOT1L, DTL, DTX3L, DUSP3, DYRK2, E2F1, E2F2, E2F4, E2F6, EEPD1, EGFR, EME1, EME2, ENDOV, EP300, EPC2, ERBB2, ERCC1, ERCC2, ERCC3, ERCC4, ERCC5, ERCC6, ERCC6L2, ERCC8, ESCO1, ESCO2, ESR1, ETS1, EXO1, EXO5, EYA1, EYA2, EYA3, EYA4, FAM175A, FAN1, FANCA, FANCB, FANCC, FANCD2, FANCE, FANCF, FANCG, FANCI, FANCL, FANCM, FBXO18, FBXO6, FEN1, FGF10, FHIT, FIGN, FIGNL1, FOS, FOXM1, FTO, FZR1, GADD45A, GADD45G, GEN1, GPS1, GSTP1, GTF2H1, GTF2H2, GTF2H2C, GTF2H3, GTF2H4, GTF2H5, H2AFX, HDAC1, HDAC2, HELQ, HERC2, HIC1, HINFP, HIST3H2A, HMGB1, HMGB2, HUS1, HUS1B, HUWE1, IFI16, IGF1, IGHMBP2, IKBKG, INIP, INO80, INO80D, INO80E, INTS3, IRS1, JMY, JUN, KAT5, KDM2A, KIAA0101, KIAA0430, KIAA2022, KIF22, KIN, KPNA2, LIG1, LIG3, LIG4, MAD2L2, MBD4, MC1R, MCM9, MCPH1, MDC1, MDM2, MDM4, MED17, MEIOB, MEN1, MGME1, MGMT, MLH1, MLH3, MMS19, MMS22L, MNAT1, MORF4L1, MORF4L2, MPG, MRE11A, MSH2, MSH3,

MSH4, MSH5, MSH6, MTA1, MUM1, MUS81, MUTYH, MYC, NABP1, NABP2, NBN, NCOA6, NEIL1, NEIL2, NEIL3, NEK1, NEK11, NFKB1, NHEJ1, NINL, NME1, NONO, NSMCE1, NSMCE2, NTHL1, NUDT1, OGG1, OTUB1, PALB2, PAPD7, PARG, PARP1, PARP2, PARP3, PARP4, PARP9, PARPBP, PCNA, PLK1, PLK3, PMS1, PMS2, PNKP, POLA1, POLB, POLD1, POLD2, POLD3, POLD4, POLDIP3, POLE, POLE2, POLE3, POLE4, POLG, POLG2, POLH, POLI, POLK, POLL, POLM, POLN, POLQ, POLR2A, POLR2B, POLR2C, POLR2D, POLR2E, POLR2F, POLR2G, POLR2H, POLR2I, POLR2J, POLR2K, POLR2L, PPM1D, PPP1CA, PPP2R2A, PPP2R5A, PPP2R5B, PPP2R5C, PPP2R5D, PPP2R5E, PPP4C, PPP4R2, PRKDC, PRMT6, PRPF19, PSMD3, PTTG1, RAD1, RAD17, RAD18, RAD21, RAD23A, RAD23B, RAD50, RAD51, RAD51AP1, RAD51B, RAD51C, RAD51D, RAD52, RAD54B, RAD54L, RAD9A, RAD9B, RASSF1, RB1, RBBP4, RBBP7, RBBP8, RBM14, RBX1, RDM1, REC8, RECQL, RECQL4, RECQL5, RELA, REV1, REV3L, RFC1, RFC2, RFC3, RFC4, RFC5, RFWD2, RFWD3, RHNO1, RNASEH2A, RNF168, RNF169, RNF8, RPA1, RPA2, RPA3, RPA4, RPAIN, RPS27A, RPS27L, RPS3, RRM2B, RTEL1, RUVBL1, RUVBL2, SETD2, SETMAR, SETX, SFPQ, SFR1, SHFM1, SHPRH, SIRT1, SIRT6, SLC30A9, SLX1A, SLX4, SMAD2, SMAD3, SMAD4, SMAD7, SMARCA1, SMARCA2, SMARCA4, SMARCA5, SMARCAD1, SMARCB1, SMARCC2, SMARCD1, SMARCD2, SMC1A, SMC2, SMC3, SMC4, SMC5, SMC6, SMG1, SMUG1, SMURF2, SOD1, SP1, SPATA22, SPIDR, SPO11, SPP1, SPRTN, SSRP1, STAT1, STRA13, SUMO1, SUPT16H, SWI5, SWSAP1, SYCP1, TAOK1, TAOK2, TAOK3, TCEA1, TDG, TDP1, TDP2, TELO2, TERF1, TERF2, TERF2IP, TEX12, TEX15, TICRR, TMEM161A, TNP1, TONSL, TOP1, TOP2A, TOP3A, TOPBP1, TP53, TP53BP1, TP73, TREX1, TREX2, TRIP12, TRIP13, TTC5, TWIST1, TYMS, UBA1, UBA52, UBB, UBC, UBE2A, UBE2B, UBE2D3, UBE2I, UBE2N, UBE2NL, UBE2T, UBE2U, UBE2V2, UBE4B, UHRF1, UIMC1,

UNG, UPF1, USP1, USP28, USP3, USP47, USP7, UVRAG, UVSSA, VCP, WDR16, WDR33, WDR48, WEE1, WHSC1, WRN, WRNIP1, WWP1, WWP2, XAB2, XPA, XPC, XRCC1, XRCC2, XRCC3, XRCC4, XRCC5, XRCC6, XRCC6BP1, YY1, ZBTB32, ZFYVE26, ZNF350, ZRANB3, ZSWIM7

Table S4.3. Primers used for Sanger validation of variants.

Gene	Variant	Forward primer	Reverse primer
AATF	c.158_159dup (p.Gly54Trpfs*157)	TGATGAAGGGGAAGATGGGG	TCTGCTCCCAATGGTCTTCA
AATF	c.755A>G (p.Asn252Ser)	TCCTGCAACTGGGGAAGATT	GGGCACTGGAAAATTCTGGG
BARD1	c.1935_1954dup (p.Glu652Valfs*69)	CCTGTAGCTGTTGAAAGGGC	TGTCAGGGGTAAAAGCATGTC
BCCIP	c.599+1G>A	AAATGACACCACCAAGCCTG	TCCCCTCACACTAAACCCAC
BLM	c.298_299del (p.Gln100Glufs*42)	CCCAACACCACAAATCAGCA	CTTTCAGAGGAGGGTGGAGG
BRCA2	c.4691dupC (p.Thr1566Aspfs*9)	AGTGACCTTCCAGGGACAAC	TAGGTGGCACCACAGTCTCA
C17orf70	c.449G>A (p.Trp150*)	CCCTATGAAGATGACGGGCT	GTCTACGAGCCAGGATGACA
CDC6	c.743del (p.Gln248Argfs*16)	AGAGCTTCCTTACGTGCTGT	TACATCATGGGGCCCTTCTC
CEP164	c.4228C>T (p.Gln1410*)	AGAGGGAGGAGGTGACAGAT	CTGTTGTGCTCATCAAGGCC
CEP164	c.1220C>T (p.Ser407Phe)	TGCCACCTTTCTAACCGTCT	TCTCAAACTCACCAGGCCAT
CHD1L	c.1086-2A>G	GCATTTGGAGGTTCTGAGGC	CATCACTCTCCTCTCGCCTT
CHD1L	c.1730G>A (p.Gly373Asp)	CAGTGTCAGTGGGATGGGAA	GTGATCGGCCCTCTTGACTA
DCLRE1A	c.C412T (p.Arg138*)	TTCAGAGCGTGGTGGAGAAT	AGCAGACCCAAGACAAGGAA
DCLRE1A	c.2575A>T (p.Ile859Phe)	GCCACAGACAACAAGAGCAT	TAATTCCAACCACAGGCAGT
DNA2	c.2493-2A>G	GCATTGGCCACTTTGTCTGA	CTTCCTCCCCTGGTGCTAAA
ENDOV	c.295C>T (p.Arg99*)	ACATCAGGTCTGGGAGTTGG	CTAGGGATGAGACACCTGCC
ENDOV	c.647G>T (p.Ser171Ile)	GTTTCTTTCCTCCAGCCGTG	ATGTCTGTGACCTGTCCTCG
ERCC6	c.2923C>T (p.Arg975*)	CAAAGACTGCCAGAACACCC	GGCCAGAAGAAGCAAGTGAC
ERCC6	c.1996C>T (p.Arg666Cys)	ATGAGCCTGGCCATCTTTCT	GTCTCTTGTAGGGGCCAGTT
FAN1	c.2128C>T (p.Arg710*)	CCTCAAAGTCCCTGTCCTGT	ACAGAGTCCACAGTAAGCCC
FAN1	c.149T>G (p.Met50Arg)	CTCAGGGTTGTCTCCTCGTT	TGTCTTTGGTGGTGGTGACT
FANCG	c.1652_1655del (p.Tyr551Phefs*7)	ACTCTAGGACACCAACTGCC	GACTCTGTACTCTGGGCTGG
FANCL	c.1096_1099dup (p.Thr367Asnfs*13)	TCGCATCATCATACCTGTCCT	GCTGACGCTTCTCCTTTATCT
HUS1	c.357+1G>A	GGAAGTTTCACGGCTTGGAA	TGGAACAGGTGAGCAAAAGC
IGHMBP2	c.1488C>A (p.Cys496*)	TCCTCCCCTACCTAAGCCTT	AGTTCCCATCACCAGACCAG
MC1R	c.67C>T (p.Gln23*)	TGGACAGGACTATGGCTGTG	TTCTCCACCAAGCTCACCAG
MC1R	c.86dup (p.Asn29Lysfs*14)	TGGACAGGACTATGGCTGTG	GGCAGCAGATGAAGCAGTAC
MC1R	c.456C>A (p.Tyr152*)	AACCTGCACTCACCCATGTA	TGAGATGCAGGAAGAAGGGG
MC1R	c.862_864del (p.Ile288del)	CCCCTTCTTCCTGCATCTCA	ATATCACCACCTCCCTCTGC
MGMT	c.593G>A (p.Trp198*)	AGCGTCACATACCACCAGAA	CATACTCAGTTTCGGCCAGC
MLH3	c.3367C>T (p.Gln1123*)	CAGCAACTTGGAGTGCAAGT	GGGTCTCAGTACAGGTGTCA
MLH3	c.1856A>T (p.Lys619Ile)	ATGTTTCTTGGGCACGTGTG	AGGAGAGTGGGCAGGATCTA
NEIL1	c.330_331insAGGC (p.Ala111Argfs*46)	CCATGGAAGGAAGCGGTTAG	CTGAAAAGAGCCGGACATGC
NEK1	c.1687_1688del (p.Ala563Tyrfs*36)	AATGCACGTGCTGCTGTACT	TGATGTACTACCCAGGAAGAGCTA

NEK1	c.2235T>G (p.Asn648Lys)	TCCAGAGGAATCACAAGTTGAC	TGAAAACCAAAAAGTAACGAGGA
NEK11	c.455+1G>A	TCTTCATTTCAGGGCCGAGA	CAAGGCGACCACACAATTCA
NINL	c.4142_4143del (p.Ser1381Cysfs*17)	CACGGAATCTAAGGCAGCAC	AGAAACAAAGCCGCCTCTTG
PARG	c.1018_1019insG (p.Lys340Argfs*11)	CCGCCCTTAGTAGAGTACCG	TAGACGTGGTGCCAAAGAGT
PARP3	c.401del (p.Lys134Argfs*33)	GAGGACTACAACTGCACCCT	TTCCTTGGCCATCTCACCTT
PMS1	c.1826G>A (p.Trp609*)	GTTCCGTTAAGCACACCCAG	AGTCACATGCAAGCTCTGGA
POLE3	c.127del (p.Val43Serfs*15)	AGTCCTTCCCTTCTCTGCAC	GGAGGCGAAGGGGAGTTTAA
POLL	c.573+1G>A	AGCAGTGATGAGTGTTGGGA	GGGGACAGATACAGGGTGAG
POLN	c.133del (p.Thr45Leufs*4)	AATGAGTGTAAAAGGGGCGG	GCTCTCCAGTGTTGCTCAGA
POLN	c.2021G>A (p.Arg674Lys)	GGATGTGACTCTGAGCAGGT	TCCTGAAGTTGGATGGGCAT
POLQ	c.2021dup (p.Lys675Glufs*16)	GATGGCCATTTGTCGATGCT	CACCTATGTTTGAGGATTGGACT
POLQ	c.7688A>G (p.Glu2563Gly)	GCTGAGGTAGGTGAAAGGGT	TCAGGGGATGGTTTGGGAAA
POLQ	c.7393G>A (p.Glu2465Lys)	GCATACCCTCTCGATGACCA	GTTGGTGTTCTGGGTTGCAT
POLQ	c.7259A>G (p.Tyr2420Cys)	GGGTTGTTCACAGTGACACA	CCTGCCCTTCTGTCTCTCTC
RFC2	c.1006C>T (p.Gln336*)	CTAAGGCGGCATTTTCCCC	ACACTCACATGAAAATAGCGGA
RHNO1	c.250C>T (p.Arg84*)	ACCAGAAACACCAAAACCGG	GCACTCTCGGATTAAGGGGA
RHNO1	c.337C>T (p.Arg113*)	TCGAAAACCTACCACCTCCA	TTCTGATTCACTGGGGCACT
RHNO1	c.45_46delinsAG (p.Leu16Val)	TGGTAGAATTGGCTGGCAGA	TGGGAAGCTGTGTAGATGCA
SMC2	c.1365_1366del (p.Arg456Thrfs*2)	CCACAGGCTCAGATGAAGTTG	AAAGCATGCTGAACCTTCCT
SPP1	c.94-1G>A	TCCGGGTGACTATATGCTTCC	GCATGATGGGCCTCTGATTG
TEX15	c.5699_5700del (p.Arg1900Asnfs*22)	AGTATCCTCCTCAAGCCCAA	GGAGCATTGTTCCGATTGCT
TEX15	c.5464T>A (p.Leu1822Ile)	CGTACTGCCTCGTTGTTCTT	TGTGCTGTTGACACTTTGGT
TEX15	c.1585_1599del (p.Ile529_Glu533del)	TTGGGAAATGTCGTGGAAGC	ACATACCCCAGGCCAAAGAA
TONSL	c.490del (p.Leu164Serfs*72)	CATGAACCGCTTCCTCATGG	GAAGTGGCTGAGTGTTTGGG
TONSL	c.2899C>T (p.Arg967Cys)	GAAGTCACCTCAGCCAACAC	TACCTGGGAATGGGAGAAGC
TONSL	c.1950C>G (p.Asp650Glu)	TGGGAGAGAGAATGCGTGTT	TGCAGAAATTGTCCGCTTCC
UBE2U	c.622C>T (p.Gln208*)	AGGTAGGTGCTCAGTCCAAA	TCTTCACTGCAGATCATGTCCT
WDR48	c.1278_1279del (p.Gly427Aspfs*8)	TGAAGATCTGGGCAAAGTGG	TCTTTTGCAGAAACCCAGGC
WRN	c.3138+2T>G	TTTCCCGTCAGCTGATCACT	AGCTTGAAGGATGAGGCTCT
WRN	c.3785C>G (p.Thr1262Arg)	ACCTCAAGAAGAACAGAAGACG	GGCCAAACTAAACTTGCTGC
ZSWIM7	c.98+1G>A	GCCTTAGCCTCCTGAGTAGC	CAGTTGACCATCACACACGG

Table S4.4. Primers used for LOH or segregation analysis of archived tissue by Sanger sequencing.

Gene	Variant	Forward primer	Reverse primer
AATF	c.158_159dup (p.Gly54Trpfs*157)	TGATGAAGGGGAAGATGGGG	TCTGCTCCCAATGGTCTTCA
AATF	c.755A>G (p.Asn252Ser)	CTTTTCAGCACTGTGGGACC	GGGCACTGGAAAATTCTGGG
BARD1	c.1935_1954dup (p.Glu652Valfs*69)	CCTGTAGCTGTTGAAAGGGC	TGTCAGGGGTAAAAGCATGTC
BRCA2	c.4691dupC (p.Thr1566Aspfs*9)	TTCATACAGCTAGCGGGAAA	AAGGTCTTTACAGGCCTCTCTG
C17orf70	c.449G>A (p.Trp150*)	CCCTATGAAGATGACGGGCT	GTCTACGAGCCAGGATGACA
CDC6	c.743del (p.Gln248Argfs*16)	AGAGCTTCCTTACGTGCTGT	GGCCTGGATACCTCTTCCTG
CHD1L	c.1730G>A (p.Gly373Asp)	GGCTGTTCTCCTGTCCACTT	GTGATCGGCCCTCTTGACTA
DCLRE1A	c.2575A>T (p.Ile859Phe)	GCCACAGACAACAAGAGCAT	TAATTCCAACCACAGGCAGT
DNA2	c.2493-2A>G	GCATTGGCCACTTTGTCTGA	CTTCCTCCCCTGGTGCTAAA
ERCC6	c.1996C>T (p.Arg666Cys)	CAACGTGCCTAACTTTCCCG	GCAGAGGAGCGTTTTAGGGT
FAN1	c.149T>G (p.Met50Arg)	CCACCTGCTAAACTTGCCTG	ATCACTTTGGCCAGGGGTTA
FANCL	c.1096_1099dup (p.Thr367Asnfs*13)	AGTTTCCAGCTCTTCACCGA	AGATTTCCTAGGTGATCTGAAACT
MGMT	c.593G>A (p.Trp198*)	TGTCTTCCAGGTCCCCATC	CATACTCAGTTTCGGCCAGC
MLH3	c.1856A>T (p.Lys619Ile)	ATGTTTCTTGGGCACGTGTG	TGCAACAACATTATGGGGAGT
NEIL1	c.330_331insAGGC (p.Ala111Argfs*46)	GTTTGTGAATGAGGCCTGCA	CTGAAAAGAGCCGGACATGC
NEK11	c.455+1G>A	GCCGAGATCTGGACGATAAA	TCCCCCAGTAGCAAATGAAC
PMS1	c.1826G>A (p.Trp609*)	AGAAACCCATGTCAGCAAGTG	AGTCACATGCAAGCTCTGGA
RHNO1	c.250C>T (p.Arg84*)	ACCAGAAACACCAAAACCGG	GCACTCTCGGATTAAGGGGA
RHNO1	c.337C>T (p.Arg113*)	TCGAAAACCTACCACCTCCA	TTCTGATTCACTGGGGCACT
RHNO1	c.45_46delinsAG (p.Leu16Val)	TGGTAGAATTGGCTGGCAGA	TGGGAAGCTGTGTAGATGCA
SMC2	c.1365_1366del (p.Arg456Thrfs*2)	CCACAGGCTCAGATGAAGTTG	TCACTACCTCCTCATGATCTGA
TEX15	c.5699_5700del (p.Arg1900Asnfs*22)	TGAGTAGCCTTTCTTCGTCCT	AAGAACAACGAGGCAGTACG
TONSL	c.490del (p.Leu164Serfs*72)	CCCCTAACTACTTCCTCCAGG	CCAGGGAGAGCTGAATGAGA
UBE2U	c.622C>T (p.Gln208*)	CAGTGCTCAAAGTTCCAAATTTCA	GGGTTTCTTCTGTTATTTCCAAGT

Table S4.5. Description of the 45 PTVs validated by Sanger sequencing, including population frequencies, familial segregation and LOH status.

Gene	Chr. Pos.	Variant (HGVS nomenclature)	Transcript Reference ID	rsID	MAF IH[a]	MAF EVS	MAF 1000G	MAF ExAC	COSMIC ID	Samples	PC segregation	LOH
AATF	chr17:35307578	c.158_159dup (p.Gly54Trpfs*157)	NM_012138.3	.	0	0	0	0		76A, 76B	Y 2/2	N, -
BARD1	chr2:215595181	c.1935_1954dup (p.Glu652Valfs*69)	NM_000465.2	.	0	0	0	0		62	-	N
BCCIP	chr10:127520177	c.599+1G>A	NM_016567.3	rs148258244	0.00087	0.00038	0	2.36E-04		34	-	-
BLM	chr15:91292792	c.298_299del (p.Gln100Glufs*42)	NM_000057.2	.	0	0	0	0		78B	Y 2/3[b,c]	-
BRCA2	chr13:32913182	c.4691dupC (p.Thr1566Aspfs*9)	NM_000059.3	.	0	7.99E-05	0	0		12	-	N
C17orf70	chr17:79518071	c.449G>A (p.Trp150*)	NM_025161.5	.	0	0	0	0		74	-	N
CDC6	chr17:38449789	c.743del (p.Gln248Argfs*16)	NM_001254.3	.	0	0	0	0		2	-	N
CEP164	chr11:117282575	c.4228C>T (p.Gln1410*)	NM_014956.4	rs147398904	0.0017	0.0016	0.0004	7.89E-04		16	-	-
CHD1L	chr1:146742591	c.1086-2A>G	NM_004284.4	.	0	0	0	0		90	Y 2/2[c]	-
DCLRE1A	chr10:115612530	c.C412T (p.Arg138*)	NM_014881.3	rs41292634	0.0048	0.0024	0.002	0.0027		59	-	-
DNA2	chr10:70182188	c.2493-2A>G	NM_001080449.2	.	0	0	0	0		64	-	N
ENDOV	chr17:78395694	c.295C>T (p.Arg99*)	NM_173627.3	rs35171431	0.00087	7.80E-05	0.0044	0.0012		83	-	-
ERCC6	chr10:50680423	c.2923C>T (p.Arg975*)	NM_000124.2	.	0	0	0	2.44E-05		53B	N 1/2	-
FAN1	chr15:31214513	c.2128C>T (p.Arg710*)	NM_014967.4	rs199845994	0	7.70E-05	0	2.44E-05		42	-	-
FANCG	chr9:35074472	c.1652_1655del (p.Tyr551Phefs*7)	NM_004629.1	.	0	0	0	0		50	Y 2/2[c,d]	-
FANCL	chr2:58386928	c.1096_1099dup (p.Thr367Asnfs*13)	NM_018062.3	.	0.0013	0.0025	0	0		47, 55B	N 1/2, -, N 1/2	N, -, -
HUS1	chr7:48018013	c.357+1G>A	NM_004507.3	.	0	0	0	2.44E-05		3A	N 1/2	-
IGHMBP2	chr11:68701332	c.1488C>A (p.Cys496*)	NM_002180.2	rs145226920	0	0.00015	0.0002	1.47E-04		39B	N 1/2	-
MC1R	chr16:89985733	c.67C>T (p.Gln23*)	NM_002386.3	rs201533137	0	0.00092	0.0014	4.01E-04		13	N 1/2	-
MC1R	chr16:89985750	c.86dup (p.Asn29Lysfs*14)	NM_002386.3	.	0.0044	0.003	0	0		69	-	-
MC1R	chr16:89986122	c.456C>A (p.Tyr152*)	NM_002386.3	rs201326893	0	0.00023	0.0002	6.67E-04		52B	Y 2/3[c,d,e,f]	N[g]
MGMT	chr10:131565137	c.593G>A (p.Trp198*)	NM_002412.3	.	0.00044	0	0	1.22E-04		63A	N 1/2	Y
MLH3	chr14:75509094	c.3367C>T (p.Gln1123*)	NM_001040108.1	rs151133595	0	0.00015	0.0004	8.95E-05		24B	N 1/2	-
NEIL1	chr15:75641315	c.330_331insAGGC (p.Ala111Argfs*46)	NM_024608.3	.	0.0017	0	0	0	COSM1683684	43	Y 2/2	N
NEK1	chr4:170428209	c.1687_1688del (p.Ala563Tyrfs*36)	NM_001199397.1	.	0	0	0	0		17, 78A	Y 2/3[c], N 1/2[b]	-, -
NEK11	chr3:130828766	c.455+1G>A	NM_024800.4	.	0	0	0	1.63E-05		68C, 68B	Y 3/4[c]	N[j]
NINL	chr20:25434092	c.4142_4143del (p.Ser1381Cysfs*17)	NM_025176.4	.	0.00044	0	0	0		72	-	N[g]
PARG	chr10:51363054	c.1018_1019insG (p.Lys340Argfs*11)	NM_003631.2	.	0	0	0	2.66E-05		47	-	-
PARP3	chr3:51978471	c.401del (p.Lys134Argfs*33)	NM_005485.4	.	0	0	0	0		33	-[f]	-
PMS1	chr2:190719824	c.1826G>A (p.Trp609*)	NM_000534.4	.	0	0	0	0		13	-	Y (alt.)
POLE3	chr9:116172359	c.127del (p.Val43Serfs*15)	NM_017443.4	.	0	0	0	0		26	-	-
POLL	chr10:103345072	c.573+1G>A	NM_013274.3	.	0	0	0	0		31B	N 1/2	-

POLN	chr4:2230817	c.133del (p.Thr45Leufs*4)	NM_181808.2	.	0	0	0	0		47	-	-
POLQ	chr3:121217455	c.2021dup (p.Lys675Glufs*16)	NM_199420.3	.	0.00044	7.99E-05	0	0		46	-	-
RFC2	chr7:73646495	c.1006C>T (p.Gln336*)	NM_181471.1	.	0	0	0	0		31B	N 1/2	-
RHNO1	chr12:2997158	c.250C>T (p.Arg84*)	NM_001252499.2	rs140887418	0.0017	0.0016	0.0008	0.0012		43	N 1/2	Y
RHNO1	chr12:2997245	c.337C>T (p.Arg113*)	NM_001252499.2	.	0	0	0	0		18	Y 2/2	N[i]
SMC2	chr9:106875703	c.1365_1366del (p.Arg456Thrfs*2)	NM_001042550.1	.	0	0	0	0		73	-	N
SPP1	chr4:88901197	c.94-1G>A	NM_000582.2	rs139555315	0.00087	0.00077	0.000599	8.46E-04		78A, 78B	Y 3/3[b,c]	-
TEX15	chr8:30700833	c.5699_5700del (p.Arg1900Asnfs*22)	NM_031271.3	.	0.0026	0.00072	0	0		24A	N 1/2	N
TONSL	chr8:145668147	c.490del (p.Leu164Serfs*72)	NM_013432.4	.	0	0	0	0		2	-	N
UBE2U	chr1:64707361	c.622C>T (p.Gln208*)	NM_152489.1	rs112337460	0.00044	0.0032	0.002	9.52E-04		14	N 1/2	-
WDR48	chr3:39125749	c.1278_1279del (p.Gly427Aspfs*8)	NM_020839.2	.	0	0	0	2.44E-05		72	-[d]	Y[k]
WRN	chr8:30999118	c.3138+2T>G	NM_000553.4	.	0	0	0	0		51	-	-
ZSWIM7	chr17:15897070	c.98+1G>A	NM_001042697.1	.	0	0	0	8.18E-06	COSM3691373	81	-	-

Chr. Pos.. chromosomal position; MAF, minor allele frequency; IH, in-house; EVS, NHLBI Exome Variant Server; 1000G, 1000 Genomes Project; ExAC, Exome Aggregation Consortium; LOH, loss of heterozygosity; Y, yes; N, no. Fraction indicates the number of carriers out of the number of PC-affected relatives tested. Alt. indicates that there is LOH of the alternate allele.

[a]In-house unaffected control exomes (n=1,045)

[b]Bi-lineal pedigree (i.e., PC diagnoses in both maternal and paternal branches of the family)

[c]Includes obligate carriers

[d]Segregation with breast cancer

[e]Segregation with skin cancer

[f]Segregation with colon cancer

[g]Absence of LOH or somatic second hit in tumour exome sequencing data

[h]Segregation with hepatocellular carcinoma

[i]Segregation with prostate cancer

[j]LOH assessed in tumour from PC-affected family member who was found to be a carrier

[k]LOH identified in tumour exome

Table S4.6. PC cases with more than 1 variant (PTV or nonsynonymous) in a putative DNA repair gene.

Sample ID	Gene	Variant
2	*CDC6*	c.743del (p.Gln248Argfs*16)
2	*TONSL*	c.490del (p.Leu164Serfs*72)
3A	*HUS1*	c.357+1G>A
3A	*TONSL*	c.2899C>T (p.Arg967Cys)
14	*MC1R*	c.67C>T (p.Gln23*)
14	*UBE2U*	c.622C>T (p.Gln208*)
16	*CEP164*	c.4228C>T (p.Gln1410*)
16	*ENDOV*	c.647G>T (p.Ser171Ile)
16	*POLN*	c.2021G>A (p.Arg674Lys)
24A	*TEX15*	c.5699_5700del (p.Arg1900Asnfs*22)
24A	*DCLRE1A*	c.2575A>T (p.Ile859Phe)
31B	*POLL*	c.573+1G>A
31B	*RFC2*	c.1006C>T (p.Gln336*)
43	*NEIL1*	c.330_331insAGGC (p.Ala111Argfs*46)
43	*RHNO1*	c.250C>T (p.Arg84*)
47	*FANCL*	c.1096_1099dup (p.Thr367Asnfs*13)
47	*PARG*	c.1018_1019insG (p.Lys340Argfs*11)
47	*POLN*	c.133del (p.Thr45Leufs*4)
53A	*CEP164*	c.1220C>T (p.Ser407Phe)
53A	*POLQ*	c.7393G>A (p.Glu2465Lys)
53B	*ERCC6*	c.2923C>T (p.Arg975*)
53B	*CEP164*	c.1220C>T (p.Ser407Phe)
68A	*POLQ*	c.7688A>G (p.Glu2563Gly)
68A	*TEX15*	c.5464T>A (p.Leu1822Ile)
72	*NINL*	c.4142_4143del (p.Ser1381Cysfs*17)
72	*WDR48*	c.1278_1279del (p.Gly427Aspfs*8)
78A	*NEK1*	c.1687_1688del (p.Ala563Tyrfs*36)
78A	*SPP1*	c.94-1G>A
78B	*BLM*	c.298_299del (p.Gln100Glufs*42)
78B	*SPP1*	c.94-1G>A
81	*ZSWIM7*	c.98+1G>A
81	*DCLRE1A*	c.2575A>T (p.Ile859Phe)
89	*MC1R*	c.862_864del (p.Ile288del)
89	*NEK1*	c.2235T>G (p.Asn648Lys)

Table S4.7. Description of the 20 missense and in-frame indels validated by Sanger sequencing, including population frequencies, predicted pathogenicity scores, familial segregation and LOH status.

Gene	Chr. Pos.	Variant (HGVS nomenclature)	Transcript Reference ID	rsID	MAF IH[a]	MAF EVS	MAF 1000G	MAF ExAC	SIFT	PolyPhen-2	CADD	GERP	COSMIC	Samples	PC segregation	LOH
AATF	chr17:35311130	c.755A>G (p.Asn252Ser)	NM_012138.3	.	0	0	0	0	0.02	0.986	23.8	5.88		32	Y 2/2[b,c]	N
CEP164	chr11:117244534	c.1220C>T (p.Ser407Phe)	NM_014956.4	rs150314805	0.00044	0.0011	0	8.05E-04	0	0.912	18.48	5.18		53A, 53B	Y 2/2	-
CHD1L	chr1:146756048	c.1730G>A (p.Gly373Asp)	NM_004284.4	rs372796148	0	7.70E-05	0	0	0.01	1	22.7	5.65		25	Y 2/2[b]	N
DCLRE1A	chr10:115602192	c.2575A>T (p.Ile859Phe)	NM_014881.3	rs11196530	0.0035	0.004	0.0018	0.0027	0.02	0.926	24.1	3.66		24A, 81	N 1/2, -	N, -
ENDOV	chr17:78399353	c.647G>T (p.Ser171Ile)	NM_173627.3	rs11558173	0	0.00024	0	1.97E-04	0	0.934	19.78	4.9		16	-	-
ERCC6	chr10:50690906	c.1996C>T (p.Arg666Cys)	NM_000124.2	rs61760163	0.0026	0.0015	0.001	0.0017	0	0.987	27.1	5.57		10	-	N
FAN1	chr15:31197015	c.149T>G (p.Met50Arg)	NM_014967.4	rs148404807	0.0013	0.0027	0.0018	0.002	0.01	0.974	22.8	5.15		58A, 58B, 34	Y 3/3[b], 2/2	-, N[d], N
MC1R	chr16:89986522	c.862_864del (p.Ile288del)	NM_002386.3	.	0	0	0	8.29E-06	.	.	.	.		89	-	-
MLH3	chr14:75514503	c.1856A>T (p.Lys619Ile)	NM_001040108.1	.	0	0	0	8.13E-06	0.01	0.925	16.23	2		8	-	Y (alt.)
NEK1	chr4:170398474	c.2235T>G (p.Asn648Lys)	NM_001199397.1	rs34324114	0.0031	0.0044	0.0016	0.0038	0.01	0.999	21.8	5.57		89	Y 2/2[b]	-
POLN	chr4:2097622	c.2021G>A (p.Arg674Lys)	NM_181808.2	.	0	0	0	0	0	1	16.16	3.68		16	-	-
POLQ	chr3:121151236	c.7688A>G (p.Glu2563Gly)	NM_199420.3	.	0.00044	0	0	1.63E-05	0	1	22.8	4.81		68A	N 1/3	-
POLQ	chr3:121155119	c.7393G>A (p.Glu2465Lys)	NM_199420.3	rs3218635	0.00044	0.00054	0	3.17E-04	0	1	32	5.81		93B, 53A	N 1/2, N 1/2	-, -
POLQ	chr3:121168167	c.7259A>G (p.Tyr2420Cys)	NM_199420.3	rs150364457	0.00044	0.00046	0.0002	3.01E-04	0	0.999	21.2	5.41		79	N 1/2	-
RHNO1	chr12:2994578	c.45_46delinsAG (p.Leu16Val)	NM_001252499.2	rs138375075, rs150099344	0.0048	0	0.0008	0.0037	0	0.999	16.25	4.44	COSM4146987	70A, 70B	Y 2/2	N, N
TEX15	chr8:30701070	c.5464T>A (p.Leu1822Ile)	NM_031271.3	rs373823105	0	7.70E-05	0	1.55E-04	0	0.999	17.18	5.54	COSM1288437	68A	N 1/3	-
TEX15	chr8:30704934	c.1585_1599del (p.Ile529_Glu533del)	NM_031271.3	.	0	8.02E-05	0	0	.	.	.	.		54	-	-
TONSL	chr8:145660507	c.2899C>T (p.Arg967Cys)	NM_013432.4	.	0	0	0	0	0	0.997	21.2	3.92		3A	N 1/2	-
TONSL	chr8:145662005	c.1950C>G (p.Asp650Glu)	NM_013432.4	rs141505364	0.00044	0.0015	0.0004	9.76E-04	0.03	0.991	17.41	2.51		86	Y 2/2[b]	-
WRN	chr8:31012237	c.3785C>G (p.Thr1262Arg)	NM_000553.4	rs78488552	0.0017	0.0035	0.0008	0.002749	0	0.974	19.22	5.48		44	Y 2/2[b]	N

Chr. Pos.. chromosomal position; MAF, minor allele frequency; IH, in-house; EVS, NHLBI Exome Variant Server; 1000G, 1000 Genomes Project; ExAC, Exome Aggregation Consortium; LOH, loss of heterozygosity; Y, yes; N, no. Fraction indicates the number of carriers out of the number of PC-affected relatives tested. Alt. indicates that there is LOH of the alternate allele.

[a]In-house unaffected control exomes (n=1,045)

[b]Includes obligate carriers

[c]Segregation with prostate cancer

[d]Absence of LOH or somatic second hit in tumour whole genome sequencing data

Table S4.8. Univariate and multivariate analysis of variables influencing survival in discovery set for all stages (n=91), early stage (n=50) and advanced stage (n=41).

					Univariate			Multivariate		
	Variables	**N**	**%**	**Mean Survival (days ± SD)**	**HR**	**95% CI**	***P* value**	**HR**	**95% CI**	***P* value**
All Stages	Age (y)	-	-	-	1.006	0.987 – 1.025	0.528	1.029	1.009 – 1.049	0.003*
	Sex									
	Male	45	49.5	814.9 (908.6)	1			1		
	Female	46	50.5	695.8 (699.7)	1.116	0.709 – 1.757	0.636	1.064	0.665 – 1.703	0.796
	Carrier Status									
	Non-carrier	60	65.9	793.4 (919.0)	1			1		
	Carrier	31	34.1	679.6 (534.9)	1.23	0.762 – 1.985	0.397	1.244	0.766 – 2.021	0.377
	Stage									
	Early (≤ IIB)	50	54.9	1077.9 (929.0)	1			1		
	Advanced (≥ III)	41	45.1	360.5 (346.0)	4.256	2.483 – 7.013	<0.001*	5.915	3.380 – 10.350	<0.001*
Early Stage (≤ IIB)	Age (y)	-	-	-	1.018	0.984 – 1.054	0.303	1.014	0.978 – 1.052	0.45
	Sex									
	Male	29	58.0	1068.5 (1038.5)	1			1		
	Female	21	42.0	1090.8 (777.5)	0.894	0.638 – 1.252	0.514	0.886	0.446 – 1.758	0.729
	Carrier Status									
	Non-carrier	30	60.0	1278.1 (1092.4)	1			1		
	Carrier	20	40.0	777.5 (496.2)	2.503	1.244 – 5.036	0.010*	2.402	1.188 – 4.853	0.015*
Advanced Stage (≥ III)	Age (y)	-	-	-	1.023	0.998 – 1.048	0.067	1.025	0.999 – 1.050	0.055
	Sex									
	Male	16	39.0	355.2 (242.3)	1			1		
	Female	25	61.0	363.9 (403.5)	1.134	0.589 – 2.188	0.704	1.373	0.698 – 2.701	0.358
	Carrier Status									
	Non-carrier	30	73.2	308.8 (197.0)	1			1		
	Carrier	11	26.8	501.6 (580.0)	0.706	0.337 – 1.479	0.356	0.748	0.359 – 1.562	0.44

Table S4.9. Description of the 39 PTVs identified in the validation WGS data. Genes in bold include those identified in the discovery set. Number of samples in the validation set (n=130) found to carry each variant is indicated.

Gene	Chr. Pos.	Variant (HGVS nomenclature)	Transcript Reference ID	rsID	MAF EVS	MAF 1000G	MAF ExAC	# Samples
AATF	chr17:35345870	c.1000C>T (p.Arg334*)	NM_012138.3	rs200484699	1.50E-04	0	2.10E-04	1
ALKBH1	chr14:78170723	c.268_281del (p.Ala90Argfs*26)	NM_006020.2	.	0	0	0	1
APEX1	chr14:20925581	c.872dup (p.Leu292Valfs*6)	NM_001641.3	.	1.60E-04	0	1.20E-04	1
ASCC3	chr6:100988113	c.5701C>T (p.Arg1901*)	NM_006828.2	.	0	0	7.90E-06	1
BLM	chr15:91328183	c.2695C>T (p.Arg899*)	NM_000057.2	.	0	0	6.30E-05	1
CHD1L	chr1:146747766	c.1386-2A>G	NM_004284.4	rs113139670	0.0011	0	8.90E-04	1
CHEK1	chr11:125514440	c.1135C>T (p.Arg379*)	NM_001274.5	.	0	0	7.90E-06	1
CRY2	chr11:45889161	c.805-2A>T	NM_021117.3	.	0	0	0	1
CYP1A1	chr15:75012998	c.1371del (p.Cys457*)	NM_000499.3	.	0.0019	6.00E-04	9.40E-04	1
CYP1A1	chr15:75013107	c.1262G>A (p.Trp421*)	NM_000499.3	.	0	0	0	1
DCLRE1A	chr10:115608826	c.2037dup (p.Leu680Alafs*13)	NM_014881.3	.	0	0	0	1
DCLRE1A	chr10:115612530	c.412C>T (p.Arg138*)	NM_014881.3	rs41292634	0.0024	0.0020	0.0027	1
FANCC	chr9:97864024	c.1642C>T (p.Arg548*)	NM_000136.2	rs104886457	7.70E-05	0	2.40E-05	1
GEN1	chr2:17955667	c.1201C>T (p.Arg401*)	NM_182625.3	.	0	0	3.20E-05	1
HERC2	chr15:28501091	c.2797_2798del (p.Leu933Serfs*8)	NM_004667.5	.	0	0	0.0046	1
IFI16	chr1:159021637	c.1666C>T (p.Arg556*)	NM_005531.2	.	0	0	0	1
MSH4	chr1:76355026	c.2198C>A (p.Ser733*)	NM_002440.3	rs149042353	2.30E-04	0	4.00E-05	1
MUM1	chr19:1367157	c.1365-2A>G	NM_032853.3	.	0	0	0	1
NBN	chr8:90947837	c.2238C>A (p.Tyr746*)	NM_002485.4	.	0	0	7.90E-06	1
NEIL2	chr8:11643738	c.955C>T (p.Gln319*)	NM_145043.2	.	0	0	7.90E-06	1
NEK1	chr4:170501992	c.868+1G>C	NM_001199397.1	.	0	0	0	1
NTHL1	chr16:2096239	c.268C>T (p.Gln90*)	NM_002528.5	rs150766139	8.50E-04	4.00E-04	0.0016	1
PNKP	chr19:50365626	c.1029+2T>C	NM_007254.3	rs199919568	8.20E-04	0	2.80E-04	1

PNKP	chr19:50365463	c.1096_1105del (p.Glu366Serfs*26)	NM_007254.3	.	0	0	0	1
POLL	chr10:103345866	c.163C>T (p.Arg55*)	NM_013274.3	rs369264701	7.70E-05	0	3.20E-05	1
POLM	chr7:44113408	c.1287_1288dup (p.Ala430Leufs*29)	NM_013284.2	.	0	0	1.60E-05	1
POLQ	chr3:121154975	c.7537C>T (p.Gln2513*)	NM_199420.3	rs148626322	7.70E-05	4.00E-04	2.10E-04	1
RECQL4	chr8:145738954	c.2200+1G>T	NM_004260.3	.	0	0	0	1
RPAIN	chr17:5326086	c.253-3_253-1del	NM_001033002.3	.	0	0	0	1
RPAIN	chr17:5326091	c.255_262del (p.Glu86Aspfs*25)	NM_001033002.3	.	0	0	0	1
RRM2B	chr8:103250867	c.184del (p.Glu62Serfs*2)	NM_001172477.1	.	0	0	1.50E-04	1
SETMAR	chr3:4354877	c.455del (p.Gly152Aspfs*22)	NM_006515.3	.	2.40E-04	0	1.60E-05	1
SHPRH	chr6:146275989	c.470del (p.Gly157Valfs*4)	NM_173082.3	.	0	0	0	1
TDP2	chr6:24667087	c.4G>T (p.Glu2*)	NM_016614.2	.	0	0	0	1
TEX15	chr8:30695397	c.7253dup (p.Leu2418Phefs*6)	NM_031271.3	.	0	0	5.50E-05	1
TEX15	chr8:30703647	c.2887C>T (p.Arg963*)	NM_031271.3	rs62000447	3.10E-04	4.00E-04	3.90E-04	1
TMEM161A	chr19:19245591	c.107+2T>G	NM_017814.2	.	0	0	9.00E-05	1
XRCC4	chr5:82554363	c.760del (p.Asp254Metfs*66)	NM_022550.2	.	0	0	0	1
XRCC6BP1	chr12:58340809	c.265G>T (p.Glu89*)	NM_033276.2	.	0	0	0	1

Chr. Pos.. chromosomal position; MAF, minor allele frequency;EVS, NHLBI Exome Variant Server; 1000G, 1000 Genomes Project; ExAC, Exome Aggregation Consortium.

Table S4.10. Univariate and multivariate analysis of variables influencing survival in validation set (n=130) of carriers versus non-carriers of PTVs in DNA repair genes identified in the discovery set (n=41) for all stages (n= 130) and early stage (n=122).

					Univariate			Multivariate		
	Variables	**N**	**%**	**Mean Survival (days ± SD)**	**HR**	**95% CI**	***P* value**	**HR**	**95% CI**	***P* value**
All Stages	Age (y)	-	-	-	1	0.980 – 1.020	0.992	1	0.980 – 1.020	0.979
	Sex									
	Male	66	50.8	722.5 (703.2)	1			1		
	Female	64	49.2	759.4 (711.0)	0.904	0.608 – 1.345	0.620	1.1	0.720 – 1.679	0.659
	Carrier Status									
	Non-carrier	120	92.3	773.1 (719.2)	1			1		
	Carrier	10	7.70	351.6 (305.1)	2.126	0.974 – 4.640	0.058	2.752	1.200 – 6.313	0.017*
	Stage									
	Early (≤ IIB)	122	93.8	768.2 (714.7)	1			1		
	Advanced (≥ III)	8	6.15	320.0 (320.7)	3.58	1.718 – 7.460	0.001*	4.165	1.911 – 9.080	<0.001*
	Family history									
	Sporadic	112	86.2	742.1 (742.8)	1			1		
	FPC	18	13.8	731.6 (404.8)	0.788	0.429 – 1.448	0.443	0.662	0.252 – 1.243	0.200
Early Stage (≤ IIB)	Age (y)	-	-	-	1.006	0.985 – 1.028	0.569	1.004	0.983 – 1.025	0.710
	Sex									
	Male	58	47.5	778.1 (724.8)	1			1		
	Female	64	52.5	759.4 (711.0)	1.02	0.675 – 1.544	0.924	1.052	0.852 – 1.300	0.636
	Carrier Status									
	Non-carrier	112	91.8	805.4 (729.6)	1			1		
	Carrier	10	82.0	351.6 (305.1)	2.631	1.252 – 5.526	0.011*	3.059	1.388 – 6.742	0.006*
	Family history									
	Sporadic	105	86.1	771.7 (753.7)	1			1		
	FPC	17	13.9	746.9 (411.9)	0.803	0.425 – 1.517	0.499	0.827	0.594 – 1.151	0.260

Table S4.11. Univariate and multivariate analyses of variables influencing survival in validation set (n=130) of carriers versus non-carriers of PTVs in any putative DNA repair gene (n=513) for all stages (n=130), early stage (n=122) and advanced stage (n=8).

					Univariate			Multivariate		
	Variables	**N**	**%**	**Mean Survival (days ± SD)**	**HR**	**95% CI**	***P* value**	**HR**	**95% CI**	***P* value**
All Stages	Age (y)	-	-	-	1.000	0.980 - 1.020	0.992	1.001	0.981 - 1.022	0.896
	Sex									
	Male	66	50.8	722.5 (703.2)	1			1		
	Female	64	49.2	759.4 (711.0)	0.904	0.608 - 1.345	0.620	1.034	0.683 - 1.566	0.867
	Carrier Status									
	Non-carrier	97	74.6	826.8 (754.7)	1			1		
	Carrier	33	25.4	487.5 (451.4)	1.706	1.073 - 2.712	0.024*	1.721	1.080 - 2.743	0.022*
	Stage									
	Early (≤ IIB)	122	93.8	768.2 (714.7)	1			1		
	Advanced (≥ III)	8	6.15	320.0 (320.7)	3.580	1.718 - 7.460	0.001*	3.823	1.762 - 8.295	0.001*
	Family history									
	Sporadic	112	86.2	742.1 (742.8)	1			1		
	FPC	18	13.8	731.6 (404.8)	0.788	0.429 - 1.448	0.443	0.779	0.423 - 1.436	0.423
Early Stage (≤ IIB)	Age (y)	-	-	-	1.006	0.985 - 1.028	0.569	1.005	0.983 - 1.026	0.674
	Sex									
	Male	58	47.5	778.1 (724.8)	1			1		
	Female	64	52.5	759.4 (711.0)	1.020	0.675 - 1.544	0.924	1.036	0.684 - 1.568	0.869
	Carrier Status									
	Non-carrier	92	75.4	850.0 (763.9)	1			1		
	Carrier	30	24.6	517.6 (462.6)	1.646	1.008 - 2.687	0.046*	1.633	0.999 - 2.669	0.051
	Family history									
	Sporadic	105	86.1	771.7 (753.7)	1			1		
	FPC	17	13.9	746.9 (411.9)	0.803	0.425 - 1.517	0.499	0.821	0.434 - 1.553	0.544

Advanced Stage (≥ III)	Age (y)	-	-	-	0.966	0.889 - 1.049	0.409	0.98	0.883 - 1.088	0.708
	Sex									
	Male	8	100	320.0 (320.7)						
	Female	0	0	0	-	-	-	-	-	-
	Carrier Status									
	Non-carrier	5	62.5	400.6 (393.5)	1			1		
	Carrier	3	37.5	185.7 (82.8)	1.939	0.387 - 9.722	0.421	1.352	0.177 - 10.324	0.771
	Family history									
	Sporadic	7	87.5	298.4 (340.0)	1			1		
	FPC	1	12.5	471.0	0.499	0.056 - 4.451	0.534	0.639	0.061 - 6.734	0.709

CHAPTER 5: General Discussion and Future Perspectives

5.1 Summary and Updates

PAC is projected to be the second leading cause of cancer death within the next decade,[15] largely owing to its advanced stage at diagnosis and lack of effective systemic therapies. While only 10-15% of PAC is thought to be attributable to inherited predisposition,[65,223,224] the characterization of germline genetic causes of PAC may provide important insights into the molecular etiology of PAC, improve risk stratification and screening strategies for at-risk individuals, guide patient selection for currently available therapies, and possibly, uncover novel therapeutic targets. A challenge in the study of PAC heredity however, is a lack of high-quality data and biospecimens, owing to the rarity and rapid lethality of the disease. To this end, we hypothesized that ***a prospective, clinic-based research registry can be used as a model to characterize the hereditary causes of PAC and identify strategies for early detection and precision oncology.***

To address these research resource needs, our lab established the Quebec Pancreas Cancer Study (QPCS), a prospective, clinic-based research registry with accompanying biospecimen repository, in April 2012. In Chapter 2, we described the rapid ascertainment methodology of the QPCS and the results following the first 374 referrals, demonstrating that a PAC research registry can be successfully implemented in a tertiary care referral center, achieving high participation rates (~90%). Further, we demonstrated that the QPCS design allows for the collection of rare "high-risk" PAC kindreds for studies of PAC heredity, including patients with a family history suggestive of FPC (2 or more affected relatives), carriers of mutations in known PAC susceptibility genes, patients with young-onset PAC (diagnosed at age 50 years or younger) and patients with ancestry known to harbour founder mutations in PAC susceptibility genes (e.g., French-Canadian ancestry).

Importantly, we show that the demographics and risk factors of patients enrolled in the QPCS are consistent with those reported in the literature.

Nearly 6 years since its inception, the QPCS now exceeds 650 probands with PAC or a related peri-ampullary condition. The inclusion of related pre-malignant and malignant peri-ampullary tumours may be important for future biomarker studies in the etiology and progression of pre-malignant lesions, as well as in studies of biomarkers to differentiate PAC from other malignancies that are difficult to distinguish with current clinical tools (e.g., imaging and biopsies). Among the now more than 400 patients with PAC enrolled in the QPCS, 59 kindreds exhibit familial clustering of PAC (~15%). This valuable resource of "high-risk" kindreds has allowed us to contribute data and samples to national and international collaborative research initiatives, including the North American Pancreatic Cancer Genetic Epidemiology (PACGENE) consortium.[147,337] This resource was essential to our studies of known PAC susceptibility genes (*BRCA1, BRCA2, PALB2* and *ATM*) in incident PAC, as well our FPC gene discovery study, which form the basis of Chapters 3 and 4 in the present thesis, respectively.

In Chapter 3, we performed comprehensive genetic analyses to define the prevalence of germline mutations in *BRCA1, BRCA2, PALB2* and *ATM* in a large consecutive series of PAC patients that were selected for FC ancestry, as well as patients that were unselected for ancestry. Among patients with FC ancestry, we observed an overall mutation rate of 7.0% (95% CI, 3.4%-13.4%) in these 4 genes. FC founder mutations predominated in this group, with an overall FC founder mutation prevalence of 5.3% (95% CI, 2.6%-10.3%). In the case series unselected for ancestry, 10.0% (95% CI, 2.7%-26.4%) of cases reporting Ashkenazi Jewish (AJ) ancestry carried an AJ founder mutation, and no non-founder mutations were identified. The mutation prevalence among patients without FC/AJ ancestry was 4.9% (95% CI, 2.6%-8.8%). We compared relevant

family history and clinical variables between carriers and non-carriers and found that carriers were more likely to be diagnosed at age 50 years or younger, and were more likely to have either 2 or more first- or second-degree relatives on the same side of the family with pancreas, breast, ovarian or prostate cancer, or 1 such relative and a second primary of one of these cancer types. Based on these findings, we propose simple criteria for reflex genetic testing, which have similar sensitivity and specificity to the current NCCN guidelines for *BRCA1* and *BRCA2* genetic testing,[367] however can be more easily applied in ambulatory settings beyond medical genetics. Finally, we provide retrospective clinical data that supports the emergent notion of a distinct clinical subtype of PAC associated with germline *BRCA1, BRCA2* and *PALB2* mutations, whereby carriers with late stage (III/IV) disease were found to have improved survival compared to non-carriers (*P*=.049), particularly if treated with platinum-based chemotherapies (*P*=.030).

Since the acceptance of our manuscript for publication, Shindo and colleagues reported a study evaluating the prevalence of germline mutations in 32 genes, including *BRCA1, BRCA2, PALB2* and *ATM,* among 854 PAC cases unselected for cancer family history.[430] Pathogenic mutations in one of these 4 genes were identified in 3.2% of unselected PAC cases,[430] with each gene contributing at frequencies consistent with the findings from our study.[402] The contribution of other known PAC susceptibility genes was low, with 2 mutations identified in *MLH1*, one each in *CDKN2A* and *TP53*, and none in the remaining MMR genes (*MSH2, MSH6, PMS2*) or HP genes (*STK11, PRSS1*).[430] Also consistent with the findings from our study, carriers were found to be diagnosed with PAC at an age significantly younger than non-carriers (60.8 ± 10.6 years versus 65.1 ± 10.1 years; *P*=0.03).[430] Notably, among mutation carriers, only 42% met NCCN criteria for *BRCA1* and *BRCA2* genetic testing.[430] Based on the age of diagnoses, ancestry and family history data provided by the authors,[430] 56% of *BRCA1, BRCA2, PALB2* and *ATM* carriers would have

been detected based on the criteria proposed in our study.[430] Unfortunately, this study did not report on the clinical outcomes of carriers.

In Chapter 4, we interrogated the germline exomes of 109 PAC cases from 93 "high risk" kindreds to search for novel PAC susceptibility genes. To nominate candidate genes, we used a filter-based approach to search for rare, protein-truncating variants (PTVs) among a list of 513 putative DNA repair genes. Rare PTVs were identified in 41 DNA repair genes in at least one kindred each. Among these genes, we searched for additional rare in-frame indels and single nucleotide variants (SNVs) that are predicted to be pathogenic by *in silico* prediction algorithms. Next, we evaluated variants for co-segregation with PAC and somatic inactivation of the wild-type allele as a means of prioritizing, but not excluding, candidate genes. Seventeen genes had stronger genetic evidence for causality, namely, PTVs in multiple kindreds, co-segregation of a variant with PAC or evidence of somatic inactivation of the wild-type allele. Several of these candidate genes warrant further investigation, however, most notable were *FAN1, NEK1* and *RHNO1*, which each had predicted-pathogenic variants in 3 kindreds (3.2%), and segregation of the variant with PAC in two kindreds. In addition, we evaluated whether there was a difference in clinical outcome, using overall survival as a surrogate, between carriers versus non-carriers of germline DNA repair gene mutations and found an adverse survival association with carrier status in early stage (stage ≤ IIB) cases, which validated in an independent series of 130 PAC cases. Overall, we conclude that there is likely considerable genetic heterogeneity in FPC, that DNA repair genes likely play an important role in PAC predisposition, and finally, that patients with germline mutations in these genes may represent a distinct clinical subtype of PAC.

During the preparation of this manuscript, we communicated our finding of *FAN1* as a candidate PAC susceptibility gene to John Rouse and colleagues, whose group first characterized

FAN1 as a DNA repair nuclease that is recruited to sites of DNA inter-strand crosslinks (ICLs) via the interaction of its ubiquitin-binding (UBZ) domain and ubiquitinated-Fancd2.[415] Interestingly, the p.Met50Arg mutation that was observed in two FPC kindreds in our study, with complete segregation of the variant with PAC,[431] occurs at a highly-conserved amino acid residue within the UBZ domain. After this communication, the group evaluated this specific mutation and found that *FAN1* p.Met50Arg mutant cells treated with mitomycin C failed to form Fan1 foci, and exhibited unrestrained replication fork progression and chromosomal instability.[432] This study functionally confirms the pathogenicity of the p.Met50Arg variant on *FAN1* function. Notably, in the same study, the group showed that 85% of mice with nuclease-defective Fan1 developed lymphomas or carcinomas by 20 months, whereas no malignancies were observed in age-matched wild-type mice, supporting a role for *FAN1* as a tumour suppressor gene.[432] These findings, as well as the finding of pathogenic *FAN1* mutations in ~3% of families meeting Amsterdam criteria for HNPCC but without identifiable germline MMR gene mutations,[417] support the putative role of *FAN1* in cancer predisposition. There were no reports of colon cancer, however, in the 3 families of *FAN1* mutation carriers from our study.

Also of note since our gene discovery study was published, is the somatic work up of the proband in Family 18, who was found to carry a PTV in *RHNO1*. This 67-year-old woman who presented with metastatic (stage IV) PAC had a complete radiological response to FOLFIRINOX (platinum-containing). She never underwent resection and had no radiological evidence of disease for 3.5 years. During this time, the patient experienced small rises in PAC tumour biomarker, CA19-9, levels that were normalized following treatment with gemcitabine. Her clinical course was so unusual for PAC that her diagnosis was questioned. We speculated that the patient's exceptional response to chemotherapy might be explained by her *RHNO1* mutation, because of its

role in activating the DNA damage response.[420] When the patient recurred with a celiac lymph node and adrenal gland metastases 3.5 years after presentation, she was resected and remains disease-free 5 years since presentation. Her lymph node metastasis was banked for research and underwent WGS. Final pathology of the metastases confirmed adenocarcinoma, consistent with PAC origin, and the WGS results also supported a diagnosis of PAC, with mutations identified in the 4 canonical PAC driver genes (*KRAS, TP53, CDKN2A* and *SMAD4*).[36-38] However, the WGS results also revealed that the patient lacked LOH or a somatic second hit in the *RHNO1* gene, and did not exhibit a genomic signature consistent with HDR deficiency (unpublished). While the absence of somatic inactivation of *RHNO1* does not support its role as a classic tumour suppressor cancer susceptibility gene, haploinsufficiency of PAC susceptibility genes has been observed, for example, in *PALB2*-associated PAC.[37,433] Further, the involvement of *RHNO1* in the DNA damage response, and not HDR directly, might explain the absence of HDR-deficient genomic features. Such genomic signatures are seldom observed in PAC associated with DNA damage response gene, *ATM*.[37,296]

The overarching conclusion of our gene discovery study in Chapter 4 is that there is likely considerable genetic heterogeneity in the remaining fraction of FPC, and that several novel DNA repair genes might be implicated, each accounting for only a small fraction of FPC. These findings are supported by two subsequent large multi-center studies in which I contributed, including a large filter-based study of germline genomes from nearly 600 FPC kindreds,[147] and a large exome-wide association study (EWAS) including 437 PAC cases and 1922 unaffected controls,[434] whereby significant genetic heterogeneity was noted in both studies, as well as a prominence of germline mutations in DNA repair genes.

In the study by Roberts and colleagues, the germline genomes of 638 patients from 593 FPC kindreds, as well as whole exomes of surgically resected PACs from 39 of these patients, were evaluated using a filter-based approach.[147] Similar to the filter-based approach used in our study, the group focused on high-quality, heterozygous, protein-truncating variants (nonsense, frameshift, splicing variants) that are present at a MAF of <0.5% in public control databases.[147] Unlike our study, however, variants of interest were limited to those seen in only a single FPC patient (i.e., "private").[147] A total of 1,077 genes contained 2 or more private heterozygous PTVs.[147] Notably, among genes with 3 or more private heterozygous PTVs in unrelated cases, 4 were known PAC susceptibility genes (*ATM, BRCA2, PALB2* and *CDKN2A*), providing validity for the overall approach, and 9 were DNA repair genes not previously implicated in PAC susceptibility (*POLN, POLQ, FANCG, BUB1B, ESCO2, FANCC, FANCM, MSH4, RAD54L*).[147] Among these, 3 DNA repair genes (*POLN*, *POLQ* and *FANCG*) were also identified in our study, with PTVs in one family each.[431] Notably, the *FANCG* variant in our study was found to co-segregate with one PAC-affected FDR and another FDR with breast cancer.[431] We also identified rare, predicted-pathogenic nonsynonymous SNVs in *POLN* (1 family) and *POLQ* (4 families), however the *POLQ* variants were found not to segregate with PAC in any of the 4 families.[431]

In a second sub-analysis, the group evaluated 87 genes that were previously known to be cancer susceptibility genes (for PAC or other cancer types) or implicated in hereditary pancreatitis, in greater detail, including large structural variant deletions (>300 bp) and rare single-nucleotide variants that were classified as pathogenic or likely pathogenic in ClinVar.[147] The frequencies of deleterious mutations in these 87 genes among FPC cases were compared to those of 967 unaffected controls from an unrelated study of patients with bipolar disorder,[435] and a statistical difference was observed only for known PAC susceptibility genes (*ATM, CDKN2A, APC, PALB2*

and *BRCA1*).[147] An additional 5 genes had *P* values between 0.05 and 0.10: *BUB1B, FANCC, BRCA2, CPA1* and *FANCG,*[147] where all genes are implicated in DNA repair except for *CPA1* which has been recently shown to predispose to hereditary pancreatitis.[436]

Based on these analyses, Roberts and colleagues proposed *BUB1B, CPA1, FANCC* and *FANCG* as top candidate PAC susceptibility genes.[147] Of these, only one variant in *BUB1B* was present in the 38 FPC kindreds for which genomes were available from multiple PAC-affected family members, and segregation with PAC was not observed.[147] Strikingly, complete segregation was *not* observed in one or more kindreds with deleterious variants in known PAC susceptibility genes, *ATM, CKDN2A, BRCA1* and *PALB2*, highlighting the prevalence of phenocopies within these FPC kindreds.[147] Finally, among the exomes of 39 PAC resection specimens from patients within their case series, a somatic mutation was observed in *BUB1B* in one tumour, however there was no associated germline mutation.[147] Previous candidate gene studies of *FANCC* and *FANCG* had insufficient evidence to support a role for these genes in PAC susceptibility.[274,275]

Among the top candidate genes identified in our study, private, heterozygous PTVs in *NEK1*, *RHNO1* and *FAN1* were reported in 3, 2, and 0 of 593 FPC kindreds in the study of Roberts and colleagues, whereas private PTVs in *NEK1* and *RHNO1* were each noted in only one of 967 controls (none significant by Fisher's exact test).[147] It should also be noted that 53 cases were overlapping between our study and the Roberts and colleagues study.

A limitation of the study by Roberts and colleagues is that only private PTVs were evaluated,[147] meaning that recurrent variants, which can result from founder effects, mutational hotspots or cryptic relatedness, were excluded from further analysis. Notably, in our study, recurrent variants were observed in 2 of our top 3 candidate genes (*NEK1*:p.Ala563Tyrfs*36 and *FAN1*:p.M50R).[431] Recurrent variants are also prevalent in known PAC susceptibility genes, for

example, the Ashkenazi Jewish *BRCA2* c.5946delT mutation.[129] Another limitation of their study is that nonsynonymous SNVs were assessed only among the 87 genes with a known role in cancer or hereditary pancreatitis, and among those, nonsynonymous SNVs were conservatively only considered deleterious if annotated as such in ClinVar, perhaps neglecting many potentially deleterious SNVs.[147] In our study, we identified candidate genes based on the presence of at least one rare PTV in that gene, and then evaluated these genes for rare, in-frame indels, as well as nonsynonymous SNVs that were predicted pathogenic by *in silico* prediction algorithms, as a means of prioritizing candidates.[431] Certainly a challenge with nonsynonymous SNVs in these studies is the inability to definitively conclude pathogenicity in the absence of functional assays, however, given the lack of a strong signal with PTVs, the inclusion of nonsynonymous SNVs may be important. Lastly, Roberts and colleagues did not evaluate noncoding variants despite the availability of whole genome data.[147]

Limitations of both our gene discovery study and that of Roberts and colleagues[147] is that family history data was retrospective and self-reported, and therefore may be incomplete or inaccurate. To overcome this challenge, there is a need for a multi-institutional prospective cohort study whereby kindred are followed longitudinally and new PAC diagnosis are ascertained as they arise in families. A second limitation of our studies is that we did not discriminate between different PAC histological subtypes (e.g., ductal and acinar cell PAC), as these data were not always unavailable, particularly in cases with metastatic disease where pathologic confirmation was limited. With advances in molecular profiling of PAC, histologic sub-classification of PAC may be facilitated, which may help to partly overcome the genetic heterogeneity that we have observed in our gene discovery studies.

In an attempt to overcome the challenges of our previous filter-based approaches, the first case-control exome-wide association study of PAC was conducted, in which we contributed.[434] The study included germline whole exome and whole genome data pooled from 5 studies, totaling 437 patients with PAC (cases) and 1922 unaffected individuals (controls).[434] It should be noted that 74 cases included in this case series were previously included in our study.[431,434] The group first evaluated rare PTVs (MAF <1%), and found *BRCA2* to have the strongest association, although not quite meeting exome-wide significance.[434] It is likely that PTVs in *BRCA2* were underrepresented in cases since known carrier status of *BRCA2* was an exclusion criteria for at least one of the studies included in this pooled case-control study.[5] No other gene had suggestive evidence (P<0.001) of an association.[434] Notably, however, cases were found to have more rare PTVs in DNA repair genes than controls (OR=1.61, P=0.005), even when excluding 13 genes already known to be PAC susceptibility genes (OR=1.35, P=0.045).[434] Among these genes, the strongest associations identified were for *POLN* and *DCLRE1A*, both of which were among the genes identified in our study,[5] and *POLN* was also described in the study by Roberts and colleagues.[147] Rare PTVs in *NEK1*, *RHNO1*, and *FAN1* were observed in 3 and 6, 3 and 9, and 2 and 3, cases and controls, respectively (none significant by Fisher's exact test).

In a second analysis that included rare nonsynonymous variants that were predicted to be pathogenic using a recently developed *in silico* prediction tool (M-CAP classifier),[437] Grant and colleagues found that there were no genes that reached exome-wide significance, however 6 genes had suggestive evidence: *UHMK1, APIG2, DNTA, CHST6, FGFR3* and *EPHA1*.[434] Notably, many of the associations reported in this study were driven by strong signals from recurrent variants,[434] again highlighting the limitations of considering only private variants in the study by Roberts and colleagues.[147]

Based on these studies, we can conclude that the genetic underpinnings of FPC is highly heterogeneous. Also consistent across studies is the observation that DNA repair genes have a significant role in FPC, with statistical evidence to support this notion.[434] These findings are not surprising given that 15 PAC susceptibility genes have been identified, collectively accounting for a small minority of FPC cases, and most implicated in DNA repair.

5.2 Challenges in Gene Discovery and Future Directions

Despite different methodologies and large sample sizes, neither the study presented in Chapter 4,[5] nor the larger collaborative studies in which we participated,[147,434] identified a major gene to explain the undefined majority of FPC, nor provided conclusive evidence for any novel PAC susceptibility gene(s). While several candidate genes were postulated, these occur at low carrier rates, and demonstrate inconsistent genetic evidence for causality (e.g., co-segregation of disease-causing alleles with PAC in FPC families and somatic inactivation of the wild-type allele in tumour specimens).

These studies highlight several challenges associated with gene discovery in FPC. First, a major conclusion from these studies is that there is considerable genetic heterogeneity in FPC. A challenge with locus heterogeneity in gene discovery is that as the number of genes implicated in FPC increases, the proportion of individuals likely to carry a disease-causing mutation in a particular gene decreases, necessitating large sample sizes. Locus heterogeneity proved a challenge even in the large multi-center study by Roberts and colleagues of nearly 600 FPC kindreds.[147]

Another challenge in studies of FPC is the presence of sporadic PAC, or "phenocopies", in FPC kindreds. Given the lack of a distinguishing clinical phenotype in hereditary or familial PAC (e.g., younger age of onset), it is not possible to identify and exclude phenocopies from these studies *a priori*. This is highlighted by the study of Roberts and colleagues where non-segregation of deleterious variants in known PAC susceptibility genes, *ATM, CDKN2A, BRCA1* and *PALB2* was observed in FPC kindreds.[147] Similarly, it is likely that many of the PAC-affected relatives in these FPC kindreds included in these studies do not share the same etiologies.

The incomplete penetrance of disease-causing alleles also poses a challenge in the interpretation of "unaffected" individuals in a kindred, as well as in the "unaffected" controls that

we often rely on for statistical analyses. Mutations in low or moderately-penetrant PAC susceptibility genes may be prevalent in these unaffected individuals.

Despite its promise as a powerful tool in gene discovery, next-generation sequencing has its own set of challenges. First, what is the best approach to distinguish disease-causing variants among the plethora of variants identified by NGS? So far, both filter-based approaches and statistical approaches have been used, each with their own advantages and disadvantages. Filter-based approaches have proven successful in the identification of PAC susceptibility genes, namely, *PALB2* and *ATM*.[136,137] While filter-based approaches allow us to narrow down vast lists of variants or genes to a smaller list of candidates, these approaches are inherently biased as they require assumptions about the prevalence of disease-causing alleles in affected individuals and the general population, as well as the pathogenicity of variants. With filter-based approaches, we risk filtering out disease-causing alleles. The likelihood of this is greater for common, low penetrance alleles.

Conversely, statistical, or "gene burden", approaches are not biased by *a priori* hypotheses, and allow investigators to consider all variants as potentially disease-causing, including variants that are common or that might have been presumed benign in filter-based approaches (e.g., nonsynsonymous variants). Statistical approaches also allow the identification of "faint signals" (i.e., common, low penetrance alleles), however there is a need for very large sample sizes of cases and controls to attain sufficient statistical power. The EWAS study by Grant and colleagues, which included 437 cases and 1922 controls, failed to identify any statistically significant associations.[434] Another challenge in statistical approaches is the need for high-quality control data, ideally that have undergone the same variant calling algorithms as cases. If indeed the remaining fraction of

FPC is due to low prevalence alleles with low penetrance effects, larger collaborative studies will be needed to obtain sufficiently powered sample sizes.

Further challenges with NGS data are the presence of sequencing artifacts, which can vary by capture kit and sequencing technology, posing an even greater challenge when data is pooled from multiple studies.

Perhaps there are alternative approaches or hypotheses that may facilitate gene discovery in FPC. With the exception of hereditary pancreatitis, there are no HPC syndromes where risk for malignancy is confined to the pancreas. Although numerous studies have demonstrated an increased risk of extrapancreatic malignancies in family members of patients with PAC (see section 1.2.4), [61,63,64,71,77,78,225,226,228-230] gene discovery studies have limited their analyses to family members with PAC. A strategy to elucidate the genetics of FPC may be to consider the syndromic nature of hereditary PAC, and extend studies of PAC heredity to include cancers that have been shown to co-occur in families with PAC.

The study of founder populations represents another untapped opportunity for gene discovery in FPC. This was recently exemplified by the discovery of *RECQL* as a novel breast cancer susceptibility gene through exome sequencing of familial or young-onset breast cancer families in two founder populations (FC and Polish).[438] As the QPCS registry continues to mature, the collection of high-risk PAC cases with FC ancestry will represent a novel avenue for gene discovery studies in FPC.

In recent years, the focus of the field has shifted from germline genetics to somatic genomics. Whereas there are no distinguishing clinical phenotypes associated with FPC or HPC, there may be distinguishing genomic phenotypes. Indeed, most of *BRCA1, BRCA2* and *PALB2*-associated PAC (due to either germline or somatic events) exhibit characteristic mutational

signatures associated with genomic instability.[37,295,296] In the studies by Waddell and colleagues[37] and Connor and colleagues,[296] only about half of the tumours exhibiting these genomic features were accounted for by mutations in *BRCA1*, *BRCA2* or *PALB2*, suggesting that there may be other genes contributing to this genomic phenotype. Whereas much of our understanding of germline genetics has been supplemented with somatic data, perhaps the reverse order of investigation – that is, studying somatic mutational data and referring back to germline data, may provide novel insights into PAC heredity.

Lastly, the study of non-coding variants and epigenetic variation at the germline level have yet to be explored in FPC.

5.3 DNA repair deficiency as a distinct clinical subtype of pancreatic cancer

The prominence of germline DNA repair gene mutations that was observed in the aforementioned studies may have important therapeutic implications, similar to what has been shown in *BRCA1, BRCA2* and *PALB2*-associated PAC treated with platinums and PARP inhibitors.[37,303,311,314] We compared the overall survival of carriers of DNA repair gene PTVs to non-carriers and found that carriers with early stage (≤IIB) disease had worse survival in multivariate analysis (HR=2.4, 95% CI 1.2-4.9, *P*=0.015).[5] There was no such difference observed for patients with advanced stage (≥III) disease.[5] This survival disadvantage observed for carriers of DNA repair gene PTVs with early stage disease was validated in an independent series of 130 PAC patients, and held when considering PTVs within the 41 candidate DNA repair genes identified in our discovery set, as well as for PTVs across all 513 putative DNA repair genes.[5] Importantly, carriers of known PAC susceptibility genes (e.g., *BRCA2*) were excluded from these analyses, since these patients may have been treated with targeted therapies and might have introduced a survival bias. A limitation of these survival analyses is that chemotherapy status was unavailable for many patients and was therefore not included as a covariate.[5] Nevertheless, the observed survival trends point to some interesting hypotheses. Could the adverse survival in patients with early stage disease suggest a more aggressive tumour biology in individuals with germline DNA repair gene mutations? Or perhaps, could carriers of DNA repair gene PTVs represent a PAC subtype with distinct therapeutic sensitivities, whereby the standard of care (i.e., Gemcitabine) is less efficacious, or even detrimental?

An important discussion point is the difference in survival outcomes observed in Chapters 3 and 4. If we hypothesize that patients with germline mutations in DNA repair genes exhibit similar clinical behavior as patients with germline *BRCA1, BRCA2* or *PALB2* mutations, how can

we explain the finding of improved survival in carriers of *BRCA1, BRCA2* and *PALB2* mutations with late stage disease (Chapter 3), but no such difference in survival in carriers of DNA repair gene PTVs with late stage disease (Chapter 4)? This might reflect differences in treatment during the times in which these cases were collected. The high-risk cases that were included in our gene discovery study were collected over more than a decade, primarily in the era in which gemcitabine monotherapy was the standard of care for advanced stage PAC. Conversely, the cases included in our HDR gene study were collected between 2014 and 2015, when advanced stage patients were more likely to receive modern chemotherapeutic regimens like FOLFIRINOX, which is platinum-containing. We can postulate that this survival advantage may be a consequence of carriers receiving platinum therapy, a notion that is supported by our finding that the survival advantage was greater when we excluded carriers that did not receive platinum-based chemotherapy. This is also in keeping with the earlier retrospective study by Golan and colleagues where patients with late stage PAC and germline *BRCA1* or *BRCA2* mutations that were treated with a platinum-containing regimen had improved survival compared to those that did not receive a platinum.[317]

When we considered all stages combined, the survival advantage of *BRCA1, BRCA2* and *PALB2* carriers was lost, however, since there were only 4 carriers with early stage disease in our study, it is difficult to draw meaningful conclusions. Notably, one of these carriers had poor survival relative to the others, and did not receive adjuvant therapy due to poor performance status.

Despite the accumulation of preclinical and retrospective clinical evidence to support the role for platinum-use in *BRCA1*, *BRCA2* and *PALB2*-associated PAC, there have been no prospective intent to treat clinical trials that have directly compared platinum-combinations to non-platinum combinations in *BRCA1* and *BRCA2*-associated PAC. Although previous small clinical trials comparing single-agent gemcitabine to gemcitabine in combination with a platinum in

advanced PAC have not independently shown significantly improved survival,[439-444] meta-analyses have suggested small but significantly improved progression-free and overall survival for platinum-based gemcitabine combinations.[445-447] *BRCA1, BRCA2* or *PALB2* carrier status was not evaluated in any of these trials, and one could hypothesize based on the aforementioned preclinical and retrospective clinical data that the observed survival benefit might be driven by subgroups of responders like patients with *BRCA1*-, *BRCA2*- or *PALB2*-deficient PAC. The increasing use of FOLFIRINOX, a platinum-containing (oxaliplatin) combination therapy, that was shown to have improved survival over gemcitabine in metastatic PAC with good performance status might in time reveal further evidence for improved outcomes in HR-deficient PAC treated with platinum combinations.[24]

Another promising opportunity for precision oncology in PAC associated with germline DNA repair gene mutations is in MMR-deficient PAC. Interestingly, the genomes of PAC tumours associated with DNA mismatch repair (MMR)- deficiency as a result of biallelic inactivation of at least one of the 4 MMR genes (*MLH1*, *MSH2*, *MSH6* and *PMS2*) have been shown to exhibit an exceptionally high mutational load, as well as increased numbers of mutation-associated neoantigens,[296] suggesting that these patients may benefit from immunotherapy. A recent phase II clinical trial assessing the efficacy of pembrolizumab (an anti-PD-1 immune checkpoint antibody) in 86 metastatic patients with MMR-deficient cancers having progressed on at least one line of chemotherapy, showed objective radiographic and complete responses in 53% and 21% of patients, respectively.[388] Notably, the study included 8 patients with PAC, all of which exhibited disease control (complete response, partial response or disease stability), which is exceptional for PAC.[388] Interestingly, the study by Connor and colleagues reported that tumour-specific

neoantigens were elevated in PAC cases with DSBR signatures, suggesting that HDR-deficient PAC may also benefit from immunotherapy approaches.[296]

5.4 Reflex genetic testing in pancreatic cancer

Given the lethality and chemoresistance of PAC, the potential to benefit the small fraction (~5%) of patients with clinically actionable germline mutations in *BRCA1, BRCA2* or *PALB2* seems to justify the costs and efforts to implement routine genetic testing (i.e., "reflex testing") for all newly diagnosed patients with PAC. A challenge, however, is that since PAC progresses rapidly, genetic testing results will need to be made available to the treating oncologist in a timely fashion for patients to benefit from optimal selection of their first-line therapy. Additional challenges associated with widespread genetic testing in PAC is the need for pre- and post-test genetic counselling, so that patients adequately understand the risks and benefits of testing for themselves and their families.[181] Most healthcare systems do not have the resources to provide this service to all incident PAC cases, and the need for genetic counselling will likely slow down the process of obtaining genetic testing results. Alternatives could be to train clinicians or nurses to provide the counselling during routine clinical care or to employ educational videos, and refer only mutation positive cases to medical genetics.[181,448] The implementation of genetic testing in the routine management of PAC will be facilitated in centers with PAC research registries, like the QPCS.

The presence of founder mutations ameliorates some of these challenges. Since only a small number of variants are being assessed, founder mutation testing is inexpensive and simple to perform, allowing rapid turnover of testing results. Genetic counselling is simplified because only mutations with confirmed pathogenic effects are tested, eliminating the challenges of variants of unknown significance (VUS). Given the simplicity of testing, and the ability of founder mutation testing to capture most carriers in these groups, it seems reasonable to offer founder mutation testing to all incident PAC cases, irrespective of family history.

Despite the potential clinical benefit, the status quo is that genetic testing in PAC is rarely pursued outside of research studies. This may in part be due to a lack of genetic testing guidelines dedicated to PAC specifically. While the American College of Gastroenterology provides some recommendations about which genes are appropriate for testing in PAC, these guidelines are limited to patients who meet criteria for FPC.[449] The NCCN guidelines for *BRCA1* and *BRCA2* genetic testing in hereditary breast and ovarian cancer includes recommendations which considers patients with PAC,[367] however in order for reflex testing to be successfully implemented in PAC, there is a need for simple, PAC-specific guidelines.

In chapter 3, we propose that reflex founder mutation screening be offered all incident patients with FC or AJ ancestry, and that reflex full gene testing of *BRCA1, BRCA2, PALB2* and *ATM* be offered to incident patients without founder ancestry or who test negative for founder mutations, and are diagnosed at age 50 years or younger, or who have a family history of 2 or more first or second-degree relatives on the same side of the family with either breast, ovarian, prostate or pancreas cancer, or 1 such relative and a personal history of one of these cancer types. These criteria will identify carriers with similar sensitivity and specificity to the NCCN guidelines, however will be simpler to apply in an ambulatory setting by surgeons and oncologists.

However, since no genetic testing guidelines have 100% sensitivity, the routine implementation of full gene sequencing and gene panels in all incident PAC may become justified as the costs of sequencing continue to fall. The challenges for genetic testing and counselling previously mentioned will be even more relevant, and additional challenges will include the interpretation of variants of unknown significance, as well as variants in genes whose roles in PAC and other cancers are less well defined.

A final consideration is whether *BRCA1, BRCA2* and *PALB2* germline carrier status is the optimal marker for drug selection. As has been discussed, PAC tumours exhibiting genomic hallmarks of HDR deficiency and instability may benefit from targeted therapies, and genomics studies have found that only about half of these tumours are accounted for by germline mutations in *BRCA1*, *BRCA2* and *PALB2*.[37,296] This implies that germline genetic testing may miss opportunities for precision oncology. As "omics" studies become more mainstream, there may be a shift from germline genetic testing to somatic genomic sequencing. Challenges associated with genomic sequencing of PAC tumours, beyond the obvious cost and time restrains, will be the need for biopsies, particularly since most patients will not undergo surgical resection. The prominent stromal reaction that is commonly associated with PAC limits tumour cellularity, requiring methods of tumour cell enrichment such as laser capture microdissection. Germline testing, on the other hand, is non-invasive, requiring only a blood sample which can be coordinated with routine clinical blood draws, is less costly and the results are simpler to interpret, making this a better option for clinical implementation in today's healthcare system.

5.5 Translational impacts of PAC research registries

In addition to the important contributions that PAC research registries have made to our current understanding of the etiology of PAC, there may be direct translational benefits for PAC-affected probands, as well as unaffected family members, who participate in these registries. For example, research registries identify individuals with clinically-relevant germline mutations who, in many cases, would not have otherwise been tested outside of research studies.[134] This may be of translational value to the patient who might subsequently be considered for precision oncology strategies, as have been described.[450] There are also benefits for unaffected "at risk" relatives, including genetic risk assessment by a genetic counsellor, education on the early signs and symptoms of PAC, modifiable risk reduction counselling (e.g., smoking cessation), and for some, inclusion in prospective research PAC screening protocols aimed at early PAC detection.[451] Family members who are found to be mutation carriers might also benefit from clinical screening programs or other preventative interventions for associated tumour spectrum malignancies, for example, breast cancer screening or prophylactic mastectomy and/or oophorectomy in *BRCA1* and *BRCA2* mutation carriers.[452]

The identification of high-risk kindreds through the QPCS has led to the establishment of a prospective PAC research screening protocol at the McGill University Health Centre for at-risk unaffected relatives enrolled in the QPCS, which involves biannual screening, alternating between endoscopic ultrasound (EUS) and magnetic resonance imaging (MRI). Similar research PAC screening protocols exist at high-volume centers in North America and Europe, which have yielded conflicting results regarding benefit.[238-250] The detection of cancers in unaffected individuals from FPC kindreds in these studies has been very low, likely because (1) not all familial clustering is hereditary in nature, (2) not all unaffected relatives in an FPC family are at risk (i.e., do not carry

the disease-causing allele) and (3) disease-causing alleles have variable penetrance. There is a need to better define the genetic causes of the remaining fraction of FPC so that more rigorous screening efforts can be implemented for individuals at highest risk.

5.6 Conclusions

Despite decades of research, progress in the detection and treatment of PAC has been incremental. In recent years, however, promising tumour subtyping with precision oncology strategies have emerged. The implication of germline mutations in both defining tumour subtypes and opportunities for targeted therapies demonstrates the translational significance of understanding the hereditary causes of PAC. In the present thesis, I have demonstrated that a research registry can serve as a dynamic resource for studies of PAC heredity. The work presented highlights the important role of DNA repair genes in PAC predisposition, with potential translational implications, including precision oncology strategies, as exemplified by *BRCA1-, BRCA2-* and *PALB2*-associated PAC, and more recently, MMR-associated PAC. While the genetic causes underlying the majority of FPC remain to be elucidated, there is much hope that collaborative efforts of PAC research registries will overcome the apparent genetic heterogeneity that has plagued gene discovery studies thus far. Further, the incorporation of "omic" data (e.g., genomic, transcriptomic, proteomic and metabolomic) into the design and analyses of clinical trials promises to further define PAC subtypes with selective therapeutic sensitivities. Advances in both generating and interpreting large "omic" datasets will facilitate such paradigm shifts.

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