Peer Review File

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Reviewer A

Comment 1:
This review is for the most part good to read and all the relevant risk factors for PC (non-genetic and genetic) are being discussed, however some more thoroughly/adequately than others. Looking at the references I get the impression that in some of the sections (especially those about genetic risk factors) not always the most relevant and/or most recent literature is being cited, for instance the BRCA1/2 references (some >20yrs old).

> Reply 1:
> We thank the reviewer for the comment. Amongst other changes, we have expounded on the segments about geography, ethnicity, gender, and pancreatitis more thoroughly, and revised the segments on *ATM* and *PALB2* to highlight the relation to hereditary breast and ovarian cancer (HBOC) syndrome that is autosomal dominant and related to pancreatic cancer rather than the rarer autosomal recessive counterparts that are not related to pancreatic cancer. These will be elaborated on and referenced in the subsequent replies. We have also updated some of the citations to be more relevant and recent. For example:
> - References 6, 8-10 on ethnic differences have been replaced with new references 6, 9-12
> - Reference 60 on Li Fraumeni has been replaced with new reference 65
> - References 70-71, 73 on *CDKN2A* have been replaced with new reference 75, 77
> - References 87-88, 90-91, 95 on *BRCA1/2* have been replaced with new references 90, 94-95

Comment 2:
Reference: page 4, line 51 to 69

The data in the geography section and the data in the ethnicity section is sometimes confusing/contradictory and needs more explanation.
For instance, the authors state that the higher PC incidence in high income countries can be associated with lifestyle risk factors (lines 46-47), but apparently there is also a high incidence of PC in African populations and these are also related to (the same) risk factors (lines 51-53) ? How can this be explained? How can African populations have the same risk factors that are also associated with higher incomes? Or are these African minority populations in higher income countries?

> Reply 2:
> We thank the reviewer for the comment and recognize that the initial manuscript was confusing in this aspect. Pancreatic cancer has a higher incidence in high income countries related to lifestyle factors. However, within high income countries where most of the studies have been conducted, there are dietary and lifestyle factors that differ between ethnicities which correlates with varying incidence of pancreatic cancer. For example, within non-Hispanic African populations in the United States, there are higher rates of smoking and obesity than that of non-Hispanic European populations. This is despite them having a generally lower income level. The new manuscript has been revised with these clarifications, and has been adjusted to compare ethnicities within the same region to avoid the confusion of

comparing populations with a different geography and ethnicity (e.g. Africans and Europeans ethnicity vs non-Hispanic Africans and non-Hispanic European ancestry). We have also included biological differences and difference in socioeconomic factors between non-Hispanic Africans and non-Hispanic Europeans to account for the difference in PC incidence.

Changes in text:
"Within a geographic region, there is a difference in the incidence of PC amongst different ethnic groups. In the United States, many studies have identified a higher incidence of PC in non-Hispanic African populations compared to non-Hispanic European populations(3, 4). Despite lower income levels amongst non-Hispanic Africans compared to non-Hispanic Europeans, differences in diet and lifestyle lead to higher rates of PC risk factors such as smoking, diabetes and obesity (5) in the non-Hispanic African group. However, a study done by Huang et al.(6) showed that non-Hispanic Africans had a 20% greater risk of PC compared to non-Hispanic Europeans even after adjusting for dietary and lifestyle differences, thus alluding to other factors at play. One such factor could be that of biological differences that cause varying susceptibility to developing PC – research suggests that the non-Hispanic Africans are slower at metabolising carcinogens from tobacco(7), and that PC in non-Hispanic Africans have increased K-ras mutations(8).

Socioeconomic factors also result in poorer overall survival for non-Hispanic Africans – a recent study has also showed that non-Hispanic Africans with PC had lower education and income level compared to non-Hispanic Europeans, and this correlated with more advanced stage at diagnosis, a lower likelihood of receiving treatment, and a longer time to commencement of treatment(9).

There have been fewer studies examining the rate of PC in other ethnic minorities, such as in Hispanic and Asian populations(6). In one US-based study, Asian populations had lower rates of smoking and obesity compared with other ethnic groups, which may contribute to their lower pancreatic cancer rates. Asian populations also have a higher survival rate compared to non-Asian populations(10), and there can be genetic factors behind this. Secreted protein acidic and rich in cysteine (SPARC), a protein that has been found to independently predict for poor disease-free survival and overall survival for patients with PC, was found by a recent study to have a lower stromal expression in Japanese patients and could be a potential factor contributing to better outcomes in this Asian population(11, 12)."

Comment 3:
Reference: Page 6 line 132

The authors attribute the difference in gender to lifestyle factors such as smoking, but in fact health differences between males and females are often better explained by their inherent difference in biology i.e. genetic differences (as advocated by prof. David Page)

Reply 3:
We have revised our manuscript to include intrinsic biological differences between the genders, such as studies that found that the female sex hormone estrogen decreases pancreatic cancer growth, another study that revealed molecular differences between tumor tissue from both male and female patients across a broad range of cancer types, and a study that found that a particular transcription factor (Kaiso) predicted for more aggressive pancreatic cancer

when found in male compared to female patients.

Changes in text:
"PC is more commonly found in males than females – this is consistent across all regions and ethnicities *(Figure 3).* The worldwide incidence of PC in 2020 is 5.7 per 100,000 for males and 4.1 per 100,000 for females(2). While the disparity could be attributed to differences in lifestyle factors, especially that of higher rates of smoking in men compared to women, there are intrinsic biological differences between the genders. Several studies suggest that the female sex hormone estrogen decreases pancreatic cancer growth(38-40), and a study using The Cancer Genome Atlas (TCGA) data has also revealed that there are distinct molecular differences between male and female patients across a broad range of cancer types(41). Another study found that Kaiso, a bi-modal transcription factor regulating gene expression, predicts for more aggressive pancreatic cancer when found in male versus female patients' tumor samples(42)."

Comment 4:
Reference: page 7 line 160

Rephrase suggestion: 10-15% has a familial and/or underlying genetic predisposition
familial = 7%
underlying (known) genetic = 3%

Reply 4: We have rephrased our sentence as suggested to make it clearer that 10-15% refers to the percentage of PC has an underlying familial and/or genetic predisposition, of which 7% are familial and 3% have an underlying known genetic predisposition.

Changes in text:
"10 to 15% of PC has a familial and/or underlying genetic predisposition, of which familial PC constitutes 7% and those with known genetic predisposition syndromes constitute 3%(51) *(Table 2)*."

Comment 5:
Reference: page 9-10 lines 206-218

This part about ATM should be revised with more focus on hereditary breast cancer instead of AT. AT is a very rare recessive disorder and far less relevant in this specific context of cancer predisposition for heterozygous carriers.
ATM is a well-known breast cancer susceptibility gene and ATM should be considered in breast cancer families, especially when pancreatic cancer is also present in these families (revise lines 215-218)

Reply 5:
We thank the reviewer for the important feedback. We have revised this segment about ATM to focus on hereditary breast cancer in heterozygous carriers, which is more relevant to the context of cancer predisposition instead of AT.

Changes in text:
"*ATM*

ATM on chromosome 11q22 codes for a protein kinase that regulates cell proliferation and detects DNA damage(68). Biallelic loss-of-function mutations of *ATM* result in Ataxia-telangiectasia (AT), a rare autosomal recessive disorder characterized by progressive ataxia, telangiectasias, immune deficiency, and increased risk of malignancies – particularly leukemias and lymphomas(69). Instead of having classic manifestations of AT, heterozygote carriers of the *ATM* mutation are at increased risk for coronary heart disease and solid organ malignancies, particularly that of breast and pancreatic cancer(70). In a study of 4607 *ATM* pathogenic variant carriers, carriers were at moderate-to-high risk for PC (OR 4.21)(71). A United Kingdom study of 1160 individuals estimated that heterozygous carriers of ATM mutation have a RR of 2.41 for developing PC(72). *ATM* is a well-established breast cancer susceptibility gene, with heterozygote carriers having more than twice the risk of the average population of developing breast cancer and a cumulative lifetime breast cancer incidence of 20-40%(73). Mutations in *ATM* should be considered in patients with PC that have a family history of breast cancer."

Comment 6:
Reference: page 10 lines 225-226

Based on their reference #73 (and many other literature), endometrial cancer is NOT an important CDKN2A associated cancer. They could mention head and neck cancers instead (larynx, pharynx, etc) as CDKN2A associated cancers (also more important than breast cancer)

Reply 6:
We thank the reviewers for the insightful feedback. We have revised the text to reflect more accurately the association of CDKN2A with head and neck and esophageal squamous cell carcinomas and non small cell lung cancers as cancers rather than breast cancers, with the relevant references.

Changes in text:
"Familial atypical multiple mole melanoma (FAMMM) is an AD condition associated with *CDKN2A* mutations, but with incomplete penetrance and variable expressivity. It is characterized by multiple atypical naevi progressing to melanoma(76), and increased risk for internal malignancies such as head and neck and esophageal squamous cell carcinomas, non small cell lung cancers and pancreatic cancer(77)."

Comment 7:
Reference: page 12 lines 267-272

this part about PALB2 is very short and as with ATM the authors do not mention anything about its well-known association with hereditary breast cancer (heterozygous mutations). In fact, PALB2 mutations should be considered in every breast cancer family and BC risks are almost comparable to BRCA1/2. Revision is needed.

Reply 7:
We thank the reviewers for the insightful feedback. We have expounded on this segment about *PALB2* being an important predisposing susceptibility gene in the development of breast cancer, and about it being a mutation that is associated with more aggressive clinicopathologic features. This is to highlight the significance of *PALB2* in the context of cancer predisposition.

Changes in text:
"*PALB2*
PALB2 on chromosome 16p12.2 encodes a protein that contributes to the cellular machinery for DNA repair by homologous recombination(94). Heterozygous mutations in carriers are significantly associated with breast cancers at an odds ratio of 3.1 to 9.2(95), which is comparable with that of *BRCA1/2*. Among the breast cancer susceptibility genes like *BRCA1/2*, *PALB2* is also considered a high penetrance gene for breast cancer. Several studies have found that *PALB2*-mutated breast cancers are associated with aggressive features, such as higher rates of triple-negative phenotype, advanced disease stage, and higher Ki67 level(96). While the prevalence of *PALB2* variants is not high, there is emerging evidence supporting *PALB2* as a susceptibility gene for PC(97). *PALB2* mutation confers a 6-fold increased PC risk(98), with a significantly earlier mean age of onset(99)."

Comment 8:
Reference: page 13 lines 292-300

the association between APC and pancreatic cancer is actually not very strong (e.g. Ghorbanoghli 2018 and Moussata 2015) and therefore revision of this section is recommended.

Reply 8:
We thank the reviewer for the important feedback. This segment on APC has been revised to reflect that while *APC* was historically been thought of as a predisposing condition for PC, more recent literature has shown that this incidence has likely been overreported in past and that the association of *APC* mutation with PC is not strong.

Changes in text:
"*APC*
APC on chromosome 5q21–22 codes for a tumor suppressor that helps to control cell proliferation, stabilize microtubules, and mediate cell migration and adhesion(105). Pathogenic variants cause Familial adenomatous polyposis (FAP), an AD syndrome classically characterized by the development of hundreds to thousands of colorectal adenomas, typically by late adolescence, which inevitably progress to colon cancer without intervention. FAP is also associated with extracolonic tumors including hepatoblastoma, duodenal, thyroid, bile duct and brain adenocarcinoma. While FAP has historically been thought of as a predisposing condition for PC(106), the incidence of classical exocrine pancreatic ductal adenocarcinoma in this population has likely been overreported in literature and we now know that the association of *APC* mutations with PC is not strong(107)."

Comment 9:
Reference: page 14 lines 314-322

This part about surveillance is misplaced because is it squeezed between sections about risk factors (familial PC/GWAS and epigenetics). If the authors want to add something about surveillance to this review paper, they should dedicate a separate section to this somewhere else in the paper.
Also note that PC surveillance is not only considered for familial PC but also for families with specific underlying gene mutations.

Reply 9:
We thank the reviewer for the comment. We have kept this section on surveillance within the segment on familial pancreatic cancer, as it was not intended to be a stand-alone section. It was intended to expound on the importance of identification and surveillance of patients with FPC due to studies showing that surveillance improves outcomes in this population. This highlights the importance of FPC as an entity. Nevertheless, we do note the reviewer's comment that it can easily come across as misplaced, and hence have clarified our sentences.

Changes in text:
"The International Cancer of the Pancreas Screening (CAPS) Consortium(115) has put forth consensus guidelines recommending that in addition to individuals with known germline mutations in susceptibility genes, individuals who are familial pancreatic cancer kindred should also undergo pancreatic surveillance to detect early pancreatic cancer and its high-grade precursors. This criteria is met by having at least one first-degree relative with pancreatic cancer who in turn also has a first-degree relative with pancreatic cancer. A 2015 systematic review by Lu et al.(116) found that PC screening in individuals with familial PC resulted in a higher curative resection rate (60% versus 25%) and longer median survival time (14.5 versus 4 months) compared with the control group. Canto et al. observed that most PCs detected during surveillance of high-risk individuals with familial PC were resectable (9 out of 10), and 85% of these patients survived for 3 years(117). This is in contrast to the general population that typically present late, with only 15 to 20% of patients being candidates for pancreatectomy(118)."

Comment 10:
Reference: page 14/15 lines 323-337

This section about epigenetics is weak and mostly irrelevant since the paper is about risk factors and PC predisposition and not about tumour biology. For instance, p16 promotor methylation is just a tumour biology mechanism and not a risk factor in itself.

Reply 10:
We thank the reviewer for the comment. After consideration, we have decided to remove the section about epigenetics as it is not very relevant to the main body of our paper.

Changes in text:
We have removed the section on epigenetics from our manuscript.

Comment 11:
Reference: Table 2 (page 26/27, line 676)

table 2 needs revision.
The authors are associating the ATM and PALB2 genes to their recessive disorders in this table but this is misleading since these recessive disorders (both very rare) are not associated with PC.
Their autosomal dominant association with hereditary breast cancer should be mentioned instead in this table.

Reply 11:
We have replaced the association of ATM and PALB2 in Table 2 to their recessive disorders

with the more prevalent and important associations with hereditary breast cancer.

Changes in text:

ATM	2. 41 (7 1)	11q22	Hereditary breast and ovarian cancer syndrome	Autosomal dominant	Multiple and early-onset breast and ovarian cancers; pancreas, prostate, melanoma and gastric cancer
PALB2	6(98)	16p12.2	Hereditary breast and ovarian cancer syndrome	Autosomal dominant	Multiple and early-onset breast and ovarian cancers; pancreas, prostate, melanoma and gastric cancer

Reviewer B:

Comment 12:
Reference: page 3, line 51-52

Line 51: "Many studies have identified a higher incidence of PC in African populations compared to European populations", the authors need to be more specific. What do they mean by African populations (non-Hispanic blacks??), and what do they mean by European populations (non-Hispanic whites??).

Reply 12:
We thank the reviewer greatly for this comment and recognize that our initial phrasing should be more specific. Our intention is to convey that non-Hispanic African populations have a higher incidence of pancreatic cancer than non-Hispanic European populations, based studies conducting within a single country i.e. the United States. The new manuscript has been revised with these clarifications, and has been adjusted to compare ethnicities within the same region (the United States) to avoid the confusion of comparing populations with a different geography and ethnicity (e.g. Africans and Europeans ethnicity vs non-Hispanic Africans and non-Hispanic European ancestry). We have also included biological differences and difference in socioeconomic factors between non-Hispanic Africans and non-Hispanic Europeans to account for the difference in PC incidence. We would like to avoid the use of the terms "black" and "white" as ethnic divisions, but rather use the terms "African" and "European" ancestry which is a better reflection of genetic background in the population described.

Changes in text:
"Within a geographic region, there is a difference in the incidence of PC amongst different ethnic groups. In the United States, many studies have identified a higher incidence of PC in non-Hispanic African populations compared to non-Hispanic European populations."

Comment 13:

Reference: page 7, line 145-158

While talking about the association between Chronic pancreatitis and PAC (line 145), I would suggest including a paragraph about the epidemiologic risk factors for patients admitted with chronic pancreatitis and pancreatic ductal adenocarcinoma in the United States. The study by Lew et al. https://www.wjgnet.com/2218-4333/full/v13/i11/907.htm

Reply 13:
We thank the reviewer for this useful comment and reference. We have expanded this section on pancreatitis to discuss the epidemiology of chronic pancreatitis – that it is more prevalent in non-Hispanic Africans, but did not translate into having a higher association of chronic pancreatitis with PC. This in turn may be attributable to socioeconomic factors contributing more restricted access to healthcare that was discussed in both the Lew et al. and Wilson et al. studies. We have also included a segment to discuss the difference in etiologies of chronic pancreatitis between non-Hispanic Africans and non-Hispanic Europeans.

Changes in text:
"Lew et al(47) found that 0.78% of patients admitted for chronic pancreatitis in a United States-based population also had PC. Blacks, men, age 40-59, and being overweight were significantly associated with chronic pancreatitis. Interesting, non-Hispanic Africans had a higher risk for chronic pancreatitis which did not translate into having a higher association of chronic pancreatitis with PC. Patients who were found to have both chronic pancreatitis and PC were predominantly non-Hispanic Europeans who were overweight and of older age. This correlated with higher incomes, better chances of getting insured and higher rates of being admitted to large urban teaching hospitals in the non-Hispanic European population.
With regards to the etiology of chronic pancreatitis, Wilcox et al.(48) reported that non-Hispanic Africans were twice as likely as non-Hispanic Europeans to be diagnosed with chronic pancreatitis attributed to alcohol or smoking, while genetic, idiopathic and autoimmune etiologies were more significant in non-Hispanic Europeans. Non-Hispanic Africans also had a longer duration of disease (8.6 versus 6.97 years) and significantly higher frequencies of severe and consistent pain, disability, and advanced pancreatic morphological changes, demonstrating different degrees of access to healthcare according to ethnicity."