Peer Review File

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Reviewer A

We thank reviewer 1 for taking the time to carefully consider our manuscript

Comment 1: Would assess TP53 for significance in association with STS (80% vs 0 for LTS); a p value is not provided but this should be feasible to determine Assess TP53 for significance in association with STS

Reply 1: Thank you, this has been completed

Changes in text: See line 173

Comment 2: Would report significance/p value for poor differentiation for its association with STS

Reply 2: Thank you, this has been completed

Changes in text: See line 168

Comment 3: The SBS17 signature in 3/4 PSC patients is particularly intriguing; would assess for significance. Recommend adding more discussion about this finding in Discussion session including potential clinical implications (might this signature ever be feasible to develop into a screening test in high risk PSC cytology samples, for example?) potential future directions for studying this finding

Reply 3: We appreciate this suggestion. We have assessed the significance of SBS17 in the PSC patients. Additional paragraphs have been added in the discussion to extensively address finding and clinical implications of SBS17. See “Discussion” paragraph 3

Changes in text: See line 194 for significance. See discussion paragraph 3

Comment 4: For the 3 patients with "locally advanced" stage: would retrospectively review records/imaging and assign TNM stage.

Reply 4: Thankyou for this, we have re-reviewed and updated these cases.

Changes in text: See “Results” paragraph 1 Line 165

Comment 5: For the 4th patient with PSC (age 75, no prior Dx) would consider re-review imaging/pathology to confirm PSC is accurate diagnosis if this hasn't been already done.

Reply 5: We agree with the reviewer regarding the unusual presentation and have re-reviewed the case and pathology reaffirms the diagnosis

Changes in text: See line 222

Reviewer B

We thank reviewer 2 for taking the time to consider our manuscript

We have addressed each of the points individually

We thank reviewer 1 for taking the time to carefully consider our manuscript

Comment 1: How to identify genetic and gene expression alterations in carcinogenesis and development of cholangiocarcinoma? What guidance can the results of this study give? It is recommended to add relevant content.

Reply 1: We appreciate the reviewers' thoughtful comments and agree that this is important. In this manuscript we have introduced and discussed results from several integrated genomic analyses (Jusakul et al., Sia et al.) and we discussed the current status of panel sequencing in PSC-CCA. Our study strengths are focussed on whole genome sequencing which gives further insights into PSC-CCA biology. We see that PSC-CCA may harbour SBS associated with hypoxia and inform prognosis. Our study suggests possible screening modalities with TP53 or WGS SBS signatures which may identify high risk patients

Changes in text: We expanded on the finding of panel sequencing in CCA. See discussion paragraph 1. We have expanded on the findings of prior integrated genomic analyses; please see discussion paragraphs 2 and 3. We discuss potential screening modalities using WGS SBS signatures or TP53; see discussion paragraph 3

Comment 2: What is the relationship between genetic and epigenetic changes and chromosomal aberrations and the development of cholangiocarcinoma? It is recommended to add relevant content

Reply 2: We thank the reviewer for this comment. Unfortunately in our study we could only look at structural and whole genomic features but have not incorporated epigenetic analyses. We have however included in the discussion (paragraph 2) the role of hypermethylation in CCA. We have

discussed the lack of chromosomal aberrations in cluster 4 and frequency of FGFR2 rearrangements in small duct CCA.

Changes in text: Expanded our discussion of epigenetic changes in CCA; see discussion paragraph 2. Discussed common chromosomal abberations in CCA; see introduction paragraph 1 and discussion paragraph 1

Comment 3: Compared with traditional methods, what is the biggest advantage of whole-genome sequencing? How to ensure its accuracy? It is recommended to add relevant content

Reply 3: Thankyou to the reviewer for highlighting this, we have added this to the discussion to highlight how signatures of SNVs and structural events are detected best by WGS and may inform prognosis

Changes in text: See discussion paragraph 5

Comment 4: How to stratify patients based on anatomical cholangiocarcinoma subtypes and genetic aberrations? What is the guiding role for the development of targeted therapies? It is recommended to add relevant content.

Reply 4: Thankyou for highlighting these important points, we have revised the introduction to discuss anatomical and histological subtypes and how they are distinctly characterised by molecular profiles which are prognostic.

Changes in text: See introduction paragraph 1

Comment 5: Please analyze the major variant characteristics of cholangiocarcinoma

Reply 5: Thankyou for this comment. We have highlighted throughout the text how anatomical, histological and molecular subtypes can vary and inform prognostic groups.

Changes in text: Please see introduction paragraph 1, Discussion paragraphs 1, 2,

Comment 6: What is the pattern and prognostic roles of TMB and immune infiltration in cholangiocarcinoma

Reply 6: Thankyou for this, we have discussed these roles in the discussion, noting that we did not perform immunophenotyping in this study

Changes in text: See Discussion paragraph 4

Comment 7: The number of patient samples in this study is too small, and a large sample study should be added for verification

Reply 7: We agree that our numbers are small, however WGS in CCA is difficult and we have highlighted this in the shortcomings of our paper

Comment 8: What are the limitations and potential solutions of WGS

Reply 8: Thank you for this comment. We have further highlighted limitations of WGS in our discussion and discussed solutions such as using LCM to enrich for cellularity or utilizing whole exome sequencing to determine molecular signatures in samples with scant cellularity.

Changes to text: Please see discussion paragraph 5