Reviewer #1:

Scientific Quality: Grade C (Good)

Language Quality: Grade C (A great deal of language polishing)

Conclusion: Accept (General priority)

Specific Comments to Authors: Sample size was still small, but their results might be informative for further studies.

We agree with the Reviewer and we had previously referred that in the discussion section of the manuscript: "Although the small sample size and lack of validation in an independent sample set significant limits the present study, our pilot data may be the basis for exploring *GNAS* methylation in larger, well-characterized sets of samples that may represent future validation studies."

Reviewer #2:

Scientific Quality: Grade D (Fair)

Language Quality: Grade A (Priority publishing)

Conclusion: Major revision

Specific Comments to Authors: In the manuscript entitled "Methylation changes at the GNAS imprinted locus in pancreatic cystic neoplasms are important for the diagnosis of malignant cysts" the authors are investigating the DNA methylation levels in GNAS locus in pancreatic cysts. The authors found methylation changes at 4 regions in this locus that were related to clinical factors. The content of the manuscript is interesting. However, there are some obvious shortage in the content of the manuscript.

Comments:

1) The impact of gender on the gene methylation level should been analyzed. The main problem of this manuscript is that the samples studied were predominantly from female patients (35/52, 67%), and the correlation should be given by correcting for age and for the sex, using a partial correlation.

As suggested, we further analysed the correlation between methylation changes and malignancy, while controlling for gender and age. We found a strong significant positive correlation between malignancy and *GNAS* methylation changes ($r=0.837$, $p<0.001$) and a moderate correlation with *GNASAS* hypermethylation ($r=0.516$, $p<0.003$) and *NESP55* hypermethylation ($r=0.497$, $p<0.006$), that was sustained after controlling for gender and age using partial correlation analysis (presented in an

additional Table – Table 3, page 21). These results were included in the Results section of the manuscript (page 11).

2) Gene methylation level may impact the gene expression status. No information of GNAS locus expression was showed in this study. It is interesting to perform some further studies to indentify the function of these GNAS transcripts in pancreatic cystic neoplasms 3) Full name of abbreviation (GNAS) should be supplied when it was first used.

Gene methylation level may impact the gene expression status but we did not study *GNAS locus* expression in this study. Specific DMR hypermethylation at the *GNAS locus* has been widely associated to *GNAS* gain of function and, more recently, somatic DNA methylation has been shown to drive transcription within the imprinted *Gnas* cluster. We agree that it would be interesting to perform further studies to identify the function of *GNAS* transcripts in pancreatic cystic neoplasms. We added this suggestion for possible future studies in a final sentence in the conclusion.

"Finally, as gene methylation may impact gene expression, additional evaluation of *GNAS* transcripts in PCF may elucidate their function in pancreatic cystic neoplasms."

3) Full name of abbreviation (GNAS) should be supplied when it was first used.

It was supplied as requested (page 6).