Author's response to reviews

Title: Primary Biliary Cirrhosis in a genetically homogeneous population: Disease associations and familial occurrence rates.

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Author's response to reviews: see over
Reviewer: Eirini Rigopoulou
Reviewer's report:
The study by Mantaka and colleagues tried to elucidate eventual lifestyle and disease associations as well as familial occurrence rates in a well geographically defined and homogeneous PBC population from Crete. This is an interesting study based on the fact, that this is the first study conducted so far in a well characterized population of PBC patients from Greece. This group had the advantage of being homogenous based on the fact that all patients were of Cretan origin and had Cretan residency. Also important was that both FDR as well as controls had the same characteristics, concerning origin and residency. There are some issues that need to be clarified further:

1) PATIENTS AND METHODS SECTION: The authors should mention in more detail methods used for determination of AMA (IIFL in which substrate, which cut-off for AMA positivity), determination of method to detect anti-M2. They also add details on the method used for the determination ANA.

Answer:
Manuscript page 7, line 12 corrected:
Antinuclear antibodies (ANA) were tested by indirect immunofluorescence on Hep-2 substrate with 1/80 cut-off of positivity. Anti-mitochondrial antibodies (AMA) and anti-smooth muscle antibodies (SMA) were tested by an indirect immunofluorescence (IIFL) assay of Nova Lite™ (IFA) on Mouse Kidney & Stomach substrate (Inova Diagnostics, San Diego CA, Inc) and a titre of ≥1/40 was considered positive, according to the manufacturer’s instructions. Anti-M2 antibodies were assessed by qualitative and quantitative ELISA (AESKULISA, German). Negative was 1-12U/ml, grey zone 12-18U/ml and positive >18U/ml.

2) RESULTS SECTION: To describe in more detail characteristics of PBC population. How many patients were AMA positive, to explain what it means AMA/M2 positive (IIFL and M2 positive??).

Answer:
Results section, page 8, line 4 corrected:
One hundred and one patients (86 females) were AMA positive in a titre ≥1/40 on IIFL with M2 higher than 18 U/ml on ELISA.

In addition the authors should mention how many patients had PBC- specific ANA and of which type.

Answer:
Added in Results section, page 8, line 6:
Forty-three of the 111 PBC patients were ANA positive, 16 with MND pattern, 5 MND and peri-nuclear, 4 peri-nuclear, 1 peri-nuclear and anti-nucleolar, 12 speckled, 1 speckled and MND, 1 speckled and anti-nucleolar and 3 diffuse.

Detailed histological data have to be also provided. To refer to progression of disease during follow up (how many died, how many were transplanted? How many had progression from stage 1-2 to stage 3-4??)
Answer:
We give more detailed histological data at the Results section, page 8, line 10-page 9, line 9:

Fourteen patients were at stage IV at diagnosis (9 on liver biopsy, and 5 AMA positive with clinical evidence of portal hypertension that did not underwent liver biopsy). Of those to the end of the study, two died after diagnosis of HCC, one with HCC is still alive and 3 are alive with de-compensated cirrhosis. Seventeen more patients were at stage III (2 AMA negative), 3 of those were stage IV alive at the end of the study, one de-compensated. Two dead (one AMA negative) of liver related causes, one within 30 days of OLT.

According to liver biopsy results at diagnosis, 79 (8 AMA negative) PBC patients were at an early stage (Ludwig I-II). During the study period three died of non liver related causes. Five are at stage IV (1 de-compensated) at the end of the study. One female AMA positive patient refused to undergo biopsy for staging.

Mild piecemeal necrosis was present in 42.35% of the biopsies, moderate in 30.6% and no piece-meal in 27.05%.

Since we have not a repeat liver biopsy in most patients we also report the progression of the Mayo score at the end of the study at page 9, line 7. The mean age and the mean Mayo score at 2010 was 64.8±11.9 years and 4.99 ± 1.46, respectively.

3) RESULTS SECTION: Table 1: The way smoking history is presented in Table 1 is rather confusing. It is not clear what the percentage in “currently smoking” represents? This should be clarified.

We modified table 1 according to both reviewers’ comments, including only specific variables and not all options.

4) RESULTS SECTION: Table 1: Percentage of patients using nail polish or hair dye is not significantly different between PBC patients and controls. They present their data as percentage, while times of exposure per year should be more accurate especially for hair dye.

Added in “Patients and Methods” (page 6, line 12) and corrected in table 1. ‘Hair dye use’ is referred as at least once a month per year, whereas ‘no hair dye use’ as never used. ‘Nail polish use’ is referred as equal or more than 10 times per year, whereas ‘no nail polish use’ is referred as occasional use or never used.

If the difference between PBC and controls is not significant, this parameter should not be included in the multivariate analysis (see page 13).

Indeed hair dye or nail polish use did not differ significantly between patients and controls in the univariate analysis, but they have been suggested as putative risk factors in previous studies (reference 25,4). We had included them in the multivariate analysis for female patients only, as mentioned in the previous manuscript in the section “Statistical analysis and in the results. But we agree with the reviewer and therefore, we removed the multivariate analysis of the female patients and present the multivariate analysis on all PBC patients including only the variables found significant in the univariate analysis.
See “statistical analysis” page 8, line 2-4 and “multivariate analysis” page 13. We also modified accordingly the discussion.

5) RESULTS SECTION: in accordance to the previous point, the authors should clarify whether parameters included in the multivariate analysis for female patients were significantly different between PBC patients and controls. This is not clear from the results presented.

Now multivariate analysis for all PBC patients includes parameters found significant in the univariate one.
See “statistical analysis” page 8, line 2-4 and “multivariate analysis” page 13. We also modified accordingly the discussion.

6) Key references (original and review articles) are missing and must be included, such as:


This very interesting reference on genetics was not on PubMed when our manuscript was submitted; we have now added it as reference 6.


We included this reference in the introduction section referring to the E coli (R 12)


This key reference already is included as reference 4 in the article.

Level of interest: An article whose findings are important to those with closely related research interests
Quality of written English: Needs some language corrections before being published
Statistical review: Yes, and I have assessed the statistics in my report.
Declaration of competing interests: I declare that I have no competing interests
Reviewer's report
Title: Primary Biliary Cirrhosis in a genetically homogeneous population: Disease associations and familial occurrence rates.

Version: 2 Date: 1 April 2012
Reviewer: Carlo Selmi
Reviewer's report:
In this manuscript, Dr. Mantaka and Colleagues report a very interesting study based on previous reports of comorbidity and risk factors for PBC. The authors utilize a series of PBC cases (115/196 currently followed) and first degree relatives (FDR) plus an equal number of unrelated controls.

Major compulsory revisions.
One cannot avoid to consider the control populations as totally unacceptable for the proposed aims. First, the unrelated control group is too small and its origin is unclear. Similarly, FDR are obviously not sex-matched and prone to numerous types of error. Lastly, the response rate among patients is significantly low.

Answer added in the 1st paragraph of Section Patients and methods in page 6, line 3:
The unrelated control group was enrolled among the visitors of the hospital. We agree that the FDR are not (and by definition can not be) age and sex related to the patients and indeed we did not directly correlate these two groups. The low response rate among patients is justified by their low educational level, rural residence (almost half of our patients) and Cretan cultural ethics and prejudices.

Minor essential revisions.
The results section is redundant and should be shortened significantly as its current form is confusing.
According to the suggestion we shortened the results section and clarified Tables.

A description of the local health system would help.
Answer added in the 1st paragraph of Section Patients and methods in page 5, line 3
The University Hospital of Heraklion is the referral centre for liver disease in the island.

The authors should justify the choice of a low alcohol intake for the definition of abuse.
ANSWER
In the text we did not use the term “abuse” but “misuse”. We chose the amount of 7 units per week for women and 14 units per week for men since this is currently the amount that is considered as “safe” from the National Institute
on Alcohol Abuse and Alcoholism (NIAA, USA). Therefore any more than that was considered as “misuse”

How did the authors determine that 55% of FDR responded to their call.

**ANSWER:** We identified siblings and children from the family history of the patients’ medical records. Moreover in the “demographics section” of the questionnaire there were questions regarding the patients’ family history, and their addresses. We therefore mailed invitation letters to 209 living FDR and 115 consented to be enrolled (consisting 55% of the living FDR).

Discretionary revision.
In the tables I would suggest including only specific variables and not all options (as in the case of BMI)
We modified tables according to both reviewers’ comments, including only specific variables and not all options.

**Level of interest:** An article whose findings are important to those with closely related research interests
**Quality of written English:** Acceptable
**Statistical review:** No, the manuscript does not need to be seen by a statistician.
**Declaration of competing interests:**
I declare that I have no competing interests.