Author's response to reviews

Title: Genetic polymorphisms located in genes related to immune and inflammatory processes are associated with end-stage renal disease: a preliminary study

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Version: 2 Date: 7 May 2012

Author's response to reviews: see over
Majadahonda, Monday, May 07, 2012

Manuscript ID: 2083131158669924

Title: Genetic polymorphisms located in genes related to immune and inflammatory processes are associated with end-stage renal disease

Author(s): María A Jiménez-Sousa, Elisabeth López, Amanda Fernández-Rodríguez, Eduardo Tamayo, Pablo Fernández-Navarro, Laura Segura-Roda, María Heredia, José I Gómez-Herreras, Jesús Bustamante, Juan M García-Gómez, Jesús F Bermejo-Martín and Salvador Resino

Dear Sir/Madam,

Thank you very much for your e-mail of April 19, 2012. Please find enclosed a point-by-point response to the reviewers’ comments and the new revised version of our manuscript. We hope that the current revised version of our manuscript is acceptable for publication. Please feel free to contact us regarding any further questions about our work.

Thank you very much for your consideration. We look forward to hearing from you at your earliest convenience.

Yours sincerely,

Salvador Resino García, PhD
Referee 1

Reviewer: Shigeru Satoh
Reviewer's report:
To editors,
This study has limitations as stated in the overall impression. As the authors mentioned, the association between the four SNPs and ESRD needs to be confirmed by replication studies with a large sample size. However, this is a well-written paper containing potentially interesting results. The methods were well described except patients. Unfortunately, I am not able to assess the statistical analysis. Finally, these preliminary results might contribute to the future studies for the prediction of the development of ESRD 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.

We want to be grateful to the referee for his/her kind appreciation about our study.

Additional comments from Referee 1:

http://www.biomedcentral.com/manuscript/review/attachment/pdf/10129095446992

The manuscript entitled "Genetic polymorphisms located genes related to immune and inflammatory processes are associated with end-stage renal disease" described that four polymorphisms, IL4R gene, CCL2 gene, STAT4 and NOS3 genes, were associated with ESRD.

Comments:
While this is a well-written paper, I have a few comments to the authors.

[Overall impression]
1) There were several limitations of this study. As the authors stated, the sample size was not large enough to detect moderate effects and the studied polymorphisms could be due to an indirect involvement of ESRD. The causes of ESRD in 276 patients were glomerulonephritis, arterial hypertension, diabetes nephropathy, tubulointestinal nephritis, polycystic kidney disease, and others. The sample size was small, however, causes of ESRD were many. A number of polymorphisms related to immune and inflammatory disease were associated with the development of ESRD in each renal disease, for example, ACEI/D gene and diabetic nephropathy. This is a preliminary study. I think "a preliminary study" is stated after the title.

Following the suggestions of the reviewer we have included the sentence "a preliminary study" in the title:

From:

Title: Genetic polymorphisms located in genes related to immune and inflammatory processes are associated with end-stage renal disease
Title: Genetic polymorphisms located in genes related to immune and inflammatory processes are associated with end-stage renal disease: a preliminary study

[Results]

1) At the first mention of the HWE and FDR, the abbreviations should be spelled out in the text as “Hardy-Weinberg equilibrium” and “false discovery rate”, respectively.

In the revised manuscript, HWE and FDR have been spelled out as “Hardy-Weinberg equilibrium” and “false discovery rate” in the text.

[Methods]

1) Didn’t they have any inflammatory or immune diseases in the control group? Why were they in the hospital?

Control subjects were selected in the hospital because of its easier accessibility. It was checked that subject did not have any inflammatory or immune disease.

Due to this point was not clearly described in the manuscript, we have performed some changes to clarify this issue in the revised manuscript.

From:

Subjects from the control-group were patients that were routinely discharged from the general medicine service with age and gender distribution similar to the case-group patients and had no evidence of renal pathology or associated diseases.

To:

Subjects from the control-group were patients who underwent to preoperative analysis prior to minor surgery at the hospital, and had no evidence of renal pathology or other immune or inflammatory diseases. Individuals were selected to have age and gender distribution similar to the case-group patients.
General Comments:
This study represented one of the recently proliferating ones, describing tentative genetic-phenotype associations from a Spanish cohort of renal transplant recipients. In general, the authors have done an admirable job in describing the baseline known information, process of recovery of the information, including the referencing to genetic databases, genetic software programs, Supplemental Tables. Good idea of separate list of important abbreviations submitted. They have, appropriately, adjusted for multiple testing (?FDR?) which removed the significance from initial positive finding, a fact perhaps somewhat under-emphasized in the paper. I think this may be important, as SNIPs were fitted here with the most likely inheritance model (so another variable introduced).
The examined index cohort of patients was heterogeneous group in terms of underlying etiology ESRD before transplant, which partially may be responsible for the lack of convincing positive findings. Thus, this study may not be a truly negative study, more likely a (relatively) failed one due to limited number of enrollees. Thus I would view the results primarily as hypothesis-generating ones.

We agree with the reviewer's comments. In order to emphasize that this is a preliminary study which can be used to generate new hypotheses in future studies, we have introduced the following sentence in the discussion:

From:
Therefore, this is a preliminary study. In future studies, the association of these SNPs with ESRD needs to be confirmed by replicating studies with a larger sample size, as well as functional studies should be performed in order to get further insights into ESRD susceptibility.

To:
Therefore, this is a preliminary study which could be considered to generate hypothesis for future studies. In fact, in further studies, the association of these SNPs with ESRD needs to be confirmed by replicating studies with a larger sample size, as well as functional studies should be performed in order to get further insights into ESRD susceptibility.

Major compulsory revisions:
Discussion.
1st sentence: so state of inflammation is inherited, acquired, or genetic predisposition in CKD?
Inflammation in CKD is probably caused by both genetic predisposition and environmental factors as many other complex diseases.
We have included a sentence clarifying the possible etiology of inflammation in CKD:

From:
It has been previously shown that biomarkers of inflammation are high even in the early stages of CKD, being linked to the risk of CKD progression to ESRD.
To:
It has been previously shown that biomarkers of inflammation are high even in the early stages of CKD. The increased inflammation could be caused by both genetic predisposition and environmental factors, being linked to the risk of CKD progression to ESR.

2nd sentence: I would not call it ?evidence? yet ? maybe a strong suggestion? especially, as all linked to pathways of inflammation (so biologically meaningful connection) but p value not significant after adjusting.

We agree with the reviewer’s recommendation. So, we have modified the second sentence of the discussion:

From: “In our study, we have found evidence of associations between ESRD and four SNPs…”
To: “In our study, we have found strong suggestion of associations between ESRD and four SNPs…”

Multiple references appear to be mislabeled and I suspect suffered corruption during the editing process (e.g. 18, 21, 22, 28, 32)

As the reviewer suspected, several references were mislabeled due to a problem with Endnote software. We have reintroduced the mislabeled references. One of the references listed by the reviewer (reference nº 24, which was previously reference nº 32) has not been modified because it was not mislabeled.

ABSTRACT:
Background.
1st paragraph ? 2nd sentence in the current format somewhat internally inconsistent, the flow of idea would work better as ?Chronic Kidney Disease progression has been linked to pro-inflammatory cytokines and markers of inflammation ? also these marker are elevated in ESRD? sequence.

We have modified the first paragraph in the background section of the abstract:

From:
End-stage renal disease (ESRD) constitutes a serious public health problem. ESRD is characterized by elevated levels of pro-inflammatory cytokines and markers of inflammation, which have been linked to the risk of chronic kidney disease progression.

To:
Chronic kidney disease progression has been linked to pro-inflammatory cytokines and markers of inflammation. These markers are also elevated in end-stage renal disease (ESRD), which constitutes a serious public health problem.

Results.
May want to add to 95% Confidence Intervals to OR, if space allows Should mention in Abstract also, hat after adjusting for multiple testing, results lost significance.
Following the suggestion of the reviewer, we have added the 95% Confidence Intervals to OR in the results section and abstract. In addition to this, we have included the sentence recommended by the reviewer (about adjusting for multiple testing).

In the results section:
From:
Significant association with ESRD was found for 4 SNPs (Table 2): AG genotype of rs1801275 in interleukin 4 receptor (IL4R) showed reduced odds of ESRD assuming an overdominant model (adjusted OR=0.66, p=0.025). In the case of rs4586 in chemokine (C-C motif) ligand 2 (CCL2), the presence of each additional copy of the minor allele was associated with reduced odds of ESRD (adjusted OR=0.70, p=0.005) in a log-additive model. On the other hand, rs301640 in an intergenic binding site for signal transducer and activator of transcription 4 (STAT4) and rs7830 in nitric oxide synthase 3 (NOS3) were associated with elevated odds of ESRD assuming a log-additive model (adjusted OR=1.82, p=0.006; and adjusted OR=1.31, p=0.043, respectively). After applying the false discovery rate (FDR) for multiple test correction, we obtained that adjusted p values for each hypothesis testing were no significant (q-value>0.05). Allelic and genotypic frequencies of significant SNPs are showed in Supplemental Table Content 2.

To:
Significant association with ESRD was found for 4 SNPs (Table 2): AG genotype of rs1801275 in interleukin 4 receptor (IL4R) gene (OR: 0.66 (95%CI=0.46-0.95); p=0.025; overdominant model), rs4586 in chemokine (C-C motif) ligand 2 (CCL2) gene (OR: 0.70 (95%CI=0.54-0.90), p=0.005) in a log-additive model. On the other hand, rs301640 located in an intergenic binding site for signal transducer and activator of transcription 4 (STAT4) and rs7830 in nitric oxide synthase 3 (NOS3) were associated with elevated odds of ESRD assuming a log-additive model (adjusted OR=1.82 (95%CI=1.17-2.83), p=0.006; and adjusted OR=1.31 (95%CI=1.01-1.71), p=0.043, respectively). After applying the false discovery rate (FDR) for multiple test correction, we obtained that adjusted p values for each hypothesis testing were no significant (q-value>0.05). Allelic and genotypic frequencies of significant SNPs are showed in Supplemental Table Content 2.

In abstract:
From:
Results: Four polymorphisms showed association with ESRD: rs1801275 in the interleukin 4 receptor (IL4R) gene (OR: 0.66; p= 0.025; overdominant model), rs4586 in chemokine (C-C motif) ligand 2 (CCL2) gene (OR: 0.70; p = 0.005; additive model), rs301640 located in an intergenic binding site for signal transducer and activator of transcription 4 (STAT4) (OR: 1.82; p= 0.006; additive model) and rs7830 in the nitric oxide synthase 3 (NOS3) gene (OR: 1.31; p= 0.043; additive model).

To:
Results: Four polymorphisms showed association with ESRD: rs1801275 in the interleukin 4 receptor (IL4R) gene (OR: 0.66 (95%CI= 0.46-0.95); p = 0.025; overdominant model), rs4586 in chemokine (C-C motif) ligand 2 (CCL2) gene (OR: 0.70 (95%CI= 0.54-0.90); p = 0.005; additive model), rs301640 located in an intergenic binding site for signal transducer and activator of transcription 4 (STAT4) (OR: 1.82 (95%CI= 1.17-2.83); p = 0.006; additive model) and rs7830 in the nitric oxide synthase 3 (NOS3) gene (OR: 1.31 (95%CI= 1.01-1.71); p = 0.043; additive model). After adjusting for multiple testing, results lost significance.
Results:
Table 1. should feature actual p values (for gender & age comparisons)

As recommended by the reviewer, we have shown the p-values for gender and age comparisons in the table 1.

| Table 1. Clinical characteristics of patients with ESRD (cases) and control group. |
|---------------------------------|-----------------|-----------------|
|                                  | Case group      | Control group   |
| No.                             | 276             | 288             |
| Age a                           | 50.0±0.78       | 52.8±1.03       | 0.030 |
| Male b                          | 108 (39.1%)     | 119 (41.3%)     | 0.573 |
| Primary disease b               |                 |                 |
| Glomerulonephritis              | 82 (29.7%)      | NA              |
| Arterial hypertension           | 30 (10.9%)      | NA              |
| Diabetic nephropathy            | 20 (7.2%)       | NA              |
| Tubulointerstitial nephritis    | 28 (10.1%)      | NA              |
| Obstructive uropathy            | 11 (4.0%)       | NA              |
| Vascular causes                 | 5 (1.8%)        | NA              |
| Polycystic kidney disease       | 37 (13.4%)      | NA              |
| Others                          | 63 (22.8%)      | NA              |

a Mean ± standard error of mean (s.e.m.). b Absolute number (percentage). NA: not applicable

Additionally, we have included a new paragraph in the discussion section:

"Another limitation of our study was that although we tried to select individuals for control-group with similar age to case-group, the comparison between means was significant (50.0 versus 52.8 years; p=0.030, Table 1). We think that these differences are so slight that have a low clinical significance. However, in order to avoid that this difference respect to age could interfere with the outcome, logistic regression analysis was adjusted by age."

Minor issues: None Level of interest: An article whose findings are important to those with closely related research interests Quality of written English: Acceptable Statistical review: Yes, but I do not feel adequately qualified to assess the statistics.

Declaration of competing interests:
I declare no competing interest