EMBO
Molecular Medicine

Manuscript EMM-2011-01127

A transcriptional network underlies susceptibility to kidney disease progression

Denise Laouari, Martine Burtin, Aurélie Phelep, Frank Bienaime, Laure-Hélène Noel, David C. Lee, Christophe Legendre, Gérard Friedlander, Marco Pontoglio, Fabiola Terzi

Corresponding author: Fabiola Terzi, INSERM U845 / Université Paris-Descartes

Review timeline:

Submission date:	07 December 2011
Editorial Decision:	18 January 2012
Editorial Correspondence	31 January 2012
Revision received:	18 April 2012
Editorial Decision:	24 April 2012
Revision received:	02 May 2012
Accepted:	07 May 2012

Transaction Report:

(Note: With the exception of the correction of typographical or spelling errors that could be a source of ambiguity, letters and reports are not edited. The original formatting of letters and referee reports may not be reflected in this compilation.)

1st Editorial Decision 18 January 2012

Thank you for the submission of your manuscript "A transcriptional network underlies susceptibility to kidney disease progression" to EMBO Molecular Medicine and please accept my sincere apologies for the late reply. We have now received reports from two out of three referees whom we asked to evaluate your manuscript. Since the review process has been lengthy we prefer to take the decision now. We will forward you the third report as soon as it becomes available.

You will see that the Reviewers find the topic of your manuscript potentially interesting. However, they also raise significant concerns on the study, which should be addressed in a major revision of the manuscript.

Importantly, Reviewer #1 highlights that the evidence supporting that Mitf-A is underlying renal lesion susceptibility mediated by the chromosome 6 locus should be strengthened. Of note, Reviewer #2 raises concerns regarding the HDAC inhibitor experiments.

Given the balance of these evaluations, we feel that we can consider a revision of your manuscript if you can convincingly address the issues that have been raised within the space and time constraints outlined below.

Revised manuscripts should be submitted within three months of a request for revision. They will otherwise be treated as new submissions, unless arranged otherwise with the editor.

I look forward to seeing a revised form of your manuscript as soon as possible.

Yours sincerely,

Editor
EMBO Molecular Medicine

***** Reviewer's comments *****

Referee #1:

The manuscript by Laouari et al is clear and well written. It describes a set of nicely performed experiments that uncover a new and possibly important mechanism involved in the progression of chronic kidney disease.

The data showing the involvement of MITFA and other components is convincing, but I am worried about how the authors arrived at this gene. They performed a genetic cross using mouse inbred strains that lead to the identification of a locus with a large confidence interval. We could argue about the method that was used to identify the locus and the confidence interval, but this has already been published in JASN and not the subject of this manuscript. Assuming this is the correct interval, it contains roughly 350 genes. In the current manuscript the others disqualified all but 23 of these genes by making the assumption that it must be a known transcription factor. After making several more assumptions in the first part of the Results section they identify MITFA.

I am not arguing that MITFA is not playing a role in the regulation of TGF-alpha and susceptibility to renal damage after nephrectomy, but I do think there is no evidence that Mitf is the gene underlying the Ckdp1 locus. To at least make it more convincing that the gene is a good candidate gene I suggest the authors add a few easy analyses to their manuscript. First, they can reduce the interval using haplotype analysis. Large parts of the QTL interval are shared between FVB, B6, and D2 and can therefore be ruled out (as there is no genetic variation). There are several online tools that can be used to perform this analysis. Second, the SNP that the authors found in the 5'UTR of the gene should segregate with the phenotype in the cross that they used to identify the QTL. The authors should genotype their G2 animals and show that this is the case.

Minor comments are:

1. I found several spelling and grammar mistakes, some typical for non-native English speakers. The authors should carefully check the text.
2. In the introduction there is a paragraph in which they cite a paper from 2006. Then the next sentence starts with "More recently, ... " followed by citations from 1999 and 2003. This is not more recent.
3. Nomenclature is a big problem. Please read the nomenclature rules for the use of mouse gene and protein names (www.informatics.jax.org/mgihome/nomen/ or http://en.wikipedia.org/wiki/Gene_nomenclature) and use the correct nomenclature rules throughout the manuscript.
4. I am a little concerned about the histology figures 1E and 1F. It might be my printer and screen, but I can not distinguish any details because of a lack in contrast.

Referee #3 (Comments on Novelty/Model System):

The findings of these new transcription regulation mechanisms in kidney disease are novel. The techniques used here are sophisticated. The experiments were well designed. The clinical relevance of these findings is high.

Referee #3 (Other Remarks):

I have pleasure to review this manuscript entitled "A transcriptional network underlines susceptibility to kidney disease progression by Laouari et al. This is well designed study with high quality of data. The findings are exciting and the conclusion is nicely supported by a large amount of data. However, I do have a few concerns here.

1) My major concern is on the findings of HDAC inhibitor. The authors found that TSA stimulates TGFa activity leading to kidney disease progression through interaction with Mitf-A. However, many previous studies suggest that HDAC inhibitors have beneficial effects on the progression of kidney disease such as anti-fibrosis effects. It would be important for authors to clarify this issue by testing the effects of TSA in these nephrectomized mouse models.
2) It would be interesting to determine whether expression of Mitf-A and Tfe3 is also regulated in human kidneys with progressive kidney disease.
3) It is well known that HIV-1 transgenic mice develop kidney disease only in FVB background. Gharavi et al have identified several HIVAN-related loci in these mice. It would be interesting to compare or discuss whether these loci overlap with the locus described here and whether these loci could interact with each other. Could Mitf-A/Tfe3 also play a role in the development of kidney disease in HIV-1 transgenic mice?
Minor: In Fig 6B, Mitf-A has double bands, what is the explanation?

Editorial Correspondence 31 January 2012

I would like to inform you that we withdrew the last reviewer from your manuscript due to the long delay.

I would thus like you to encourage to revise the text according to the reports you already received and look forward to reading your revised manuscript.

Please don't hesitate to contact me if you have any inquiries.

Yours sincerely,

Editor
EMBO Molecular Medicine

1st Revision - Authors' Response 18 April 2012

We thank the two Reviewers for their thoughtful comments. We feel that the revised version of the manuscript has been considerably improved by their inputs.

Referee 1

Referee 1 suggested to try to improve the evidence that the *Mitfa* variant is genetically involved in our phenotype. He claimed that " … *First, they can reduce the interval using haplotype analysis. Large parts of the QTL interval are shared between FVB, B6, and D2 and can therefore be ruled out (as there is no genetic variation). There are several online tools that can be used to perform this analysis. Second, the SNP that the authors found in the 5'UTR of the gene should segregate with the phenotype in the cross that they used to identify the QTL. The authors should genotype their G2 animals and show that this is the case".*

We agree with the Referee on the need to improve our analysis. We decided to use the Perlegen Mouse SNP Browser (http://mouse.cs.ucla.edu/perlegen/). The results showed that our *Ckdp1* confidence interval is heavily fragmented (117 fragments) in its ancestral origin. Overall, 25 Mb have been found identical between the sensitive and at least one of the two resistant strains over a total of 37 Mb (17-cM). On the other hand, there are 11 Mb that are ancestrally different between the FVB/N and the resistant strains. Of note, *Mitfa* is in a haplotype block that is different between the sensitive and resistant strains. We observed that 15 over the 23 candidate genes encoding for transcription factors were in haplotype regions that are conserved between the sensitive and resistant strains.

The idea of looking at the G/A variant segregation is an excellent one. When we analyzed the G/A variant in all the G2 progeny, we found that the rs3066317 was the marker that segregated with the

highest concordance between the observed and the expected phenotype. Remarkably, this variant corresponded to the lowest P value of the *Ckdp1* locus. These data are summarized in Table S2 of supporting information.

We agree with all the "minor comments" raised by Referee 1.

1. "*I found several spelling and grammar mistakes, some typical for non-native English speakers*"

   In the new version, the text has been revised by a native English speaker.

2. *"In the introduction there is a paragraph in which they cite a paper from 2006. Then the next sentence starts with "More recently, ..." followed by citations from 1999 and 2003. This is not more recent"*.

   We apologize for this mistake. The sentence has been corrected.

3. *"Nomenclature is a big problem. Please read the nomenclature rules for the use of mouse gene and protein names (www.informatics.jax.org/mgihome/nomen/ or http://en.wikipedia.org/wiki/Gene_nomenclature) and use the correct nomenclature rules throughout the manuscript"*.

   The names of genes and proteins have been corrected according to the international approved rules.

4. *"I am a little concerned about the histology figures 1E and 1F. It might be my printer and screen, but I can not distinguish any details because of a lack in contrast."*

   The quality of Figures 1E and 1F have been improved in the revised version of the manuscript.

Referee 3

Referee 3 recognizes that our work is a "*... well designed study with high quality of data. The findings are exciting and the conclusion is nicely supported by a large amount of data."*

However, he/she has a few concerns.

1. *"My major concern is on the findings of HDAC inhibitor. The authors found that TSA stimulates TGFa activity leading to kidney disease progression through interaction with Mitf-A. However, many previous studies suggest that HDAC inhibitors have beneficial effects on the progression of kidney disease such as anti-fibrosis effects. It would be important for authors to clarify this issue by testing the effects of TSA in these nephrectomized mouse models"*.

   We agree with the Referee that it has been shown that TSA treatment improve the progression of renal lesions in some experimental models of chronic kidney disease. However, in my opinion, given the complexity of the pathological mechanisms involved, it is relatively difficult to interpret the molecular mechanisms of this beneficial effect. Histone acetylation has been shown to control the expression of a huge number of genes in different cell types. An impressively large number of molecular cascades implying either positive or negative regulators will be modulated by this treatment. In fact, it has been reported that at least 20% of known genes are up or down-regulated by a treatment with HDAC inhibitors. Just to mention an example of this complexity in our experimental model, we have observed that TSA treatment significantly increased TGF-_ promoter activity in MITF-A cotransfected cells. However, previous studies (*Deribe YL, Sci Signal 2009, 2:ra84; Chou CW, Plos One 2011, 6:e18087; Zhou Q, Brest Cancer Res Trat 2009, 117:443; Bruzzese F, J Cell Physiol 2011, 226:2378*), including one in diabetic nephropathy (*Gilbert RE, Kidney Int 2011, 79:1312*), have reported that HDAC inhibitors hamper EGFR signaling pathway activation, by downregulating the EGFR expression and favoring its endocytosis. For all these reasons, I would rather avoid any direct speculation against or in favor of our hypothesis involving a drug with such a vast pleiotropic effect.

2. *"It would be interesting to determine whether expression of Mitf-A and Tfe3 is also regulated in human kidneys with progressive kidney disease"*.

   We thank the referee for this suggestion that allowed us to extend our results to human CKD. As suggested, we studied MITF-A and TFE3 expression in a cohort of renal transplant recipients.

We chose this group because renal transplant patients are a human model of nephron number reduction and in our center, they systematically undergo a surveillance renal transplant biopsy one year post-transplantation. Remarkably, we observed that whereas TFE3 staining markedly increased in tubular nuclei of damaged kidneys, MITF-A, when expressed, was predominantly found in tubular nuclei of kidneys with normal morphology. These data are illustrated in the new Figure 8.

3. "*It is well known that HIV-1 transgenic mice develop kidney disease only in FVB background. Gharavi et al have identified several HIVAN-related loci in these mice. It would be interesting to compare or discuss whether these loci overlap with the locus described here and whether these loci could interact with each other. Could Mitf-A/Tfe3 also play a role in the development of kidney disease in HIV-1 transgenic mice?"*.

   As requested by the referee, we compared the *Ckdp1* locus with the loci that have been previously reported to predispose the FVB/N strain to more severe HIVAN. We have also compared our locus to other susceptibility loci that have been shown to impair diabetic nephropathy and congenital nephrotic syndrome in FVB/N mice. Interestingly, all these loci map to distinct chromosomal regions, suggesting that different molecular pathways might underlie different forms of CKD. On the other hand, since each locus has been linked to a different renal phenotype, we cannot rule out the possibility that different genetic networks trigger these distinct events. These observations are now discussed in the revised version of the manuscript.

4. *"Minor: In Fig 6B, Mitf-A has double bands, what is the explanation?"*

   It has been described that MITF-A is subject to various post-translational modifications, i.e. phosphorylation or sumoylation. In our experiments, depending on the running time and the resolution of the polyacrylamide gels used in western-blot, a double band can be observed that most likely reflects different levels of MITF-A post-translational modifications.

2nd Editorial Decision 24 April 2012

Thank you for the submission of your revised manuscript "A transcriptional network underlies susceptibility to kidney disease progression" to EMBO Molecular Medicine. We have now received the enclosed reports from the referees that were asked to re-assess it. You will be glad to see that the reviewers are now globally supportive and we can proceed with official acceptance of your manuscript pending the changes detailed below.

Please modify the manuscript text as highlighted by reviewer #1.

On a more editorial note, the description of all reported data that includes statistical testing must state the name of the statistical test used to generate error bars and P values, the number (n) of independent experiments underlying each data point (not replicate measures of one sample), and the actual P value for each test (not merely 'significant' or 'P < 0.05') (please see http://onlinelibrary.wiley.com/journal/10.1002/(ISSN)1757-4684/homepage/ForAuthors.html#data2 for more information). In addition, please include a Table of Contents as the first page of the Supplementary Material file and combine all Supporting Information in one PDF file. Also, immunoblots should be surrounded by a black line to indicate the borders of the blot, if the background is faint.

Please submit your revised manuscript within two weeks. I look forward to seeing a revised form of your manuscript as soon as possible.

Yours sincerely,

Editor
EMBO Molecular Medicine

***** Reviewer's comments *****

Referee #1:

I am happy to see the incorporation of my suggestion on testing the Mitf variant in the new version of the manuscript, but do not understand why they did take up my suggestion of the haplotype analysis and say in the response that it does strengthen their results but then do not mention it in the manuscript.

Other minor comment that I missed in my first review of the abstract: MITF_A is not a protective modifier. It is a modifier, but whether it is protective or not depends on the allele. If one allele is protective then the other is not. Therefore it should say "we identified MITF-A, ..., as a modifier of CKD progression.
Referee #3:

I am satisfied by the revision. No further comments

2nd Revision - Authors' Response 02 May 2012

Point-by-point response to reviewer comments

Referee 1

We agree with the Referee that the haplotype analysis strengthens our genetic study. As requested, these data have now been included in the first paragraph of the Results section.

We also agree that, depending of the allele, MITF-A can be either a protective or a deleterious modifier. The sentence has been corrected.

