Author's response to reviews

Title: Anti-glomerular basement membrane glomerulonephritis and thrombotic microangiopathy in first degree relatives; a case report

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Version: 2 Date: 2 July 2012

Author's response to reviews: see over
Dear Dr Henderson,
Executive Editor at *BMC Nephrology*

Thank you for the review of our manuscript and for the opportunity to resubmit to *BMC Nephrology*. We are grateful for your consideration of publication of our manuscript as a case report. The manuscript has been revised according to the referees’ comments and we believe that the manuscript has been much improved.

The revised manuscript conforms to the journal manuscript preparation guidelines; however, we will be pleased to assist if you or the referees need supplementary information. All authors have read and approved the final version of the revised manuscript.

Yours sincerely,
Thomas Idorn, MD, PhD Student

**Authors’ response to the referees’ comments**

Below we have responded to each of the referees’ comments with a point-by-point description including:

1. The referee comment
2. The authors’ response
3. Changes that have been made:

   If minor changes have been made due to the referee comment, we have shown the paragraph / section that have been changed both in the original version (red letters) and the revised version (blue letters).

   A new paragraph / minor section that have been added to the manuscript due to the referee comments are also shown.

We have uploaded a version of the manuscript in which we have underlined any change that we have made and a final version of the revised manuscript.
Referee 1, Christine Skerka, Introduction:
In this study Idorn and coauthors present a case report involving a women diagnosed with anti-glomerular basement membrane glomerulonephritis and her daughter with pregnancy-induced thrombotic microangiopathy. The investigators expected a similar genetic background or factor in both related patients which predispose the patients to end stage renal disease. Interestingly the relatives developed different diseases and the identification of one predisposing factor in the related patients would explain the pathomechanism that is involved in both these rare kidney diseases. Although the investigators sequenced several candidate genes and performed complement assays, they did not succeed to find a relevant predisposing factor in the patients. Despite this fact the investigations represent valuable data for the clinical community, as they describe the coincidence of two rare kidney diseases TMA and Goodpasture’s disease in one family. The mutations or polymorphisms in several tested genes are obviously not associated with disease pathology in this family.

Authors’ response:
We thank the referee for the interest in our research field, for the work that she has put into reviewing this manuscript and we agree with her reflections.

- Referee 1, minor corrections, comment 1.1:
Please mark the histology pictures. Thromboses were? fibrin? fragmented erys? Crescentic formations and so on.

Authors’ response:
We agree with the referee that marking of the histological characteristics directly in the pictures will compromise the Figure legends and the case report in general.

Original version, legend Figure 1 p.14:
Light microscopy, x200, hematoxylin and eosin stain. Thrombosis of the afferent arteriole and partial necrosis of the glomerulus with deposition of fibrin and fragmented erythrocytes.

Revised version, legend Figure 1 p.14:
Light microscopy, x200, hematoxylin and eosin stain. Thrombosis of the afferent arteriole (†, large arrow) and partial necrosis of the glomerulus with deposition of fibrin (*) and fragmented erythrocytes (†, small arrow).

Figure 1 has been marked accordingly to the figure legend in the revised version of the manuscript.

*Original version, legend Figure 2 p.14:*

Light microscopy, x200, hematoxylin and eosin stain. Diffuse extracapillary glomerulonephritis with predominant fresh crescentic formations and a few older crescentic formations with fibrosis. Compression of the preserved part of glomeruli.

*Revised version, legend Figure 2 p.14:*

Light microscopy, x200, hematoxylin and eosin stain. Diffuse extracapillary glomerulonephritis with predominant fresh crescentic formations (*) and a few older crescentic formations with fibrosis (**). Compression of the preserved part of glomeruli (†).

Figure 2 has been marked accordingly to the figure legend in the revised version of the manuscript.

*Referee 2, Karl Lhotta, Introduction:*

This is an interesting report of two rare renal diseases occurring in the same family. Although the authors tried their best to identify a possible link in the pathogenesis of the two diseases, the overall result of this effort is rather inconclusive. This fact is correctly stated in the abstract and in the discussion.

Authors’ response:

We thank the referee for the interest in our research field, for the work that he has put into reviewing this manuscript and for the acknowledgement of our effort to identify a common link between the two diseases.

- *Referee 2, major comment 1:*

The report does not contain information on the C3 levels of the patients. C3 is frequently reduced in aHUS, and low levels would strengthen this diagnosis.

Authors’ response:
We agree that measurement of low C3 levels would strengthen the diagnosis, whereas a normal level would not exclude the diagnosis. C3 levels were not measured and unfortunately no further testing is feasible. The information is now included.

Revised version, p.6: Measurement of C3 levels were not performed

- Referee 2, major comment 2:
  One possible explanation is that the daughter also had an autoantibody-mediated disease. Unfortunately anti ADAMTS13 antibodies were not determined at time of acute illness. Anti factor H antibodies should also be considered as a possibility.

Authors’ response:
We agree that anti factor H antibodies could play a role as well. These were not measured in the initial diagnostic workup. In the follow up we have looked for deletions involving CFHR1 which is strongly associated with anti factor H antibodies and did not find any deletions. This information is now included. Further analyses are not feasible.

Revised version, p.6: CFH-autoantibodies were not investigated, but none of patients were carriers of the common CFHR1/CFHR3 deletion or other CFHR1 deletions strongly associated with antibody induced aHUS [Zipfel et al 2010].

- Referee 2, major comment 3:
A family tree would be helpful. Were there signs of kidney disease in other family members?

Authors’ response:
We agree with the referee’s assertion that kidney diseases in other family members should be mentioned. We therefore contacted the daughter for further elaboration of the family history of kidney disease. The daughter did a great effort to help us and contacted several relatives in order to ensure the family history of kidney disease. No other family members have ever experienced kidney disease. Since we only have two cases, we decided not to include a family tree in the case report; however, we would be able to do so on request from the referee or the Editor.
Original version, p.5:

Additional analyses

Following maternal diagnosis, the following analyses were done:

**HLA tissue typing...**

Revised version, p.5:

Additional analyses

Following maternal diagnosis, additional information was obtained and the following analyses were done:

**Family history:** Further elaboration of the family history of kidney disease was done in order to identify additional cases. No other family members had ever experienced clinically significant kidney diseases or any signs of kidney disease. It was not possible to obtain blood samples for genetic analyses from the father. Mother and daughter lived in the same household until a few years before disease onset of the daughter. There were no obvious exposures to environmental factors such as drugs, hydrocarbons or other toxins. They discharged different occupations.

**HLA tissue typing...**

- **Referee 2, major comment 4:**
  Did the two patients live in the same household? Could there be a common environmental factor such as exposure to a toxin?

**Authors’ response:**
This is a relevant question, since especially anti-GBMGN is known to be induced by environmental factors, including hydrocarbons. Mother and daughter did live in the same household until a few years before disease onset of the daughter, but otherwise there were no suspicious and/or common exposure to toxins or other environmental factors. They discharged different occupations.
Changes in the manuscript due to this comment are incorporated in the answer to referee 2, major comment 3, previously mentioned.

**Referee 3, Fadi Fakhouri, Introduction:**
Idorn and colleagues report on the occurrence of pregnancy-related TMA and anti-GBM glomerulonephritis in a female patient and her biological mother, respectively. The occurrence of two relatively rare renal disorders in the same family is peculiar, even though in rare cases (discussed by the authors), TMA may complicate the course of anti-GBM disease. The authors failed to document a common pathogenic mechanism underlying both disorders. The patient with pregnancy-TMA had a Factor I mutation, previously reported in MPGN and aHUS. However, the functional consequences of this mutation have not been proven.

Authors’ response:
We thank the referee for the interest in our research field, for the work that he has put into reviewing this manuscript and we agree with his reflections.

**Referee 3, comment 1:**
The main message of the case report is rather of limited importance and a "Letter to the Editor" format may be more suitable for this report.

Authors’ response:
We agree with the referee that the main message of the case report could also be reported in a ‘Letter to the Editor’ format. However, we believe that the present case involves a substantial amount of interesting clinical observations and genetic analyses, which would be difficult to summarize in a ‘Letter to the Editor’ format. Since the Editor, Dr Valenzuela, did not ask us to change the format in the response letter, we have kept the ‘Case report’ format.

**Referee 4, Kostas Stylianou, Introduction:**
This is an interesting paper regarding two cases, a mother with anti-glomerular basement membrane glomerulonephritis and her daughter who presented with thrombotic microangiopathy 14 years earlier. The authors tried to connect the two cases by seeking a common genetic defect, focusing on the complement proteins. The daughter was found to be heterozygous carrier of the CFI G261D
mutation, yet the mother was not. They also found a common polymorphism of the complement factor H which is associated with increase susceptibility for aHUS but not for anti-GBMGN. The manuscript is well written but there are some major concerns.

Authors’ response:
We thank the referee for the interest in our research field, for the work that he has put into reviewing this manuscript and we agree with his reflections.

- Referee 4, major comment 1:
The authors state that the G261D mutation has no functional effect on complement regulation. A citation is needed for this statement. I suspect they mean that the mutation does not affect the plasma CFI levels as happened with the two cases with CFI mutation reported by Servais et al (ref 9). Did they measure CFI levels?

Authors’ response:
We agree that the reference for this statement is missing and it has now been included; In 2007 Nilsson et al. reported on three unrelated patients with aHUS, where genetic testing on CFH, MCP and CFI revealed the G261D mutation in all three patients and no other mutations. Despite extensive in vitro studies no effect on complement regulation could be found. The mutated protein retained its complement inhibitory functions, the mutation had no effect on factor I serum levels, and the mutation did not affect mRNA splicing or protein glycosylation.

Since thorough investigation of the mutation was previously performed, we did not investigate this further. Normal factor I serum levels was also found in the patient reported by Servais et al. We did not measure factor I serum levels in our patients and further analyses are not feasible.

Original version, p.7:
No functional effect of the G261D mutation on complement regulation has been demonstrated. However, it has been found in other patients with C3 glomerulonephritis and aHUS [9] and may have effects not revealed by in vitro tests, or be a marker for a linked genetic deficiency.

Revised version, p.7:
The G261D mutation has been found in other patients with C3 glomerulonephritis and aHUS [9+Nilsson et al. 2007]. Despite several tests by Nilsson et al, no functional effect of the G261D mutation on factor I mediated complement regulation or factor I serum levels has been demonstrated [Nilsson et al. 2007], however, it may have effects not revealed by the in vitro tests, or be a marker for a linked genetic deficiency.

- Referee 4, major comment 2:
Servais et al (9) reported on two CFI mutations (A222G and G243D). I wonder whether the G243D mutation is the same with the present G261D mutation.

Authors’ response:
This is correct, and has now been highlighted in the text as follows:

*Original version, p.6:*
Gene sequencing for CFH, CFI, MCP and thrombomodulin showed heterozygosity for a CFI mutation (G261D)

*Revised version, p.6:*
Gene sequencing of *CFH, CFI, MCP* and *THBD* showed heterozygosity for a *CFI* mutation (G261D, alternative syntax G243D)

- Referee 4, major comment 3:
It would be interesting to look at other family members (e.g the father) for haematuria or history of kidney disease. Since the mother was not a carrier of the mutation, the father should at least be heterozygous. Therefore, if the father has some kind of kidney pathology (and especially C3 glomerulopathy) we could come up to the conclusion that the G261D mutation does have some impact. If he’s not affected, then it is indeed possible that the mutation has no functional effect.

Authors’ response:
This is a relevant point from the referee. As mentioned earlier (referee 2, major comment 3 and 4), we did do further elaboration of the family history of kidney diseases. Unfortunately we did not
have the opportunity to do further investigations of the father since he passed away several years ago.

Response to this comment is incorporated in the answer to referee 2, major comment 3, previously mentioned.

- Referee 4, major comment 4:
The authors state the daughter’s renal biopsy did not show any deposits but only thrombotic microangiopathy lesions. Lack of C3 deposits makes complement dysregulation a less likely explanation for the pathogenesis of aHUS; even more so, if the G261D mutation has no functional effect. Please comment.

Authors’ response:
The daughter’s biopsy was examined with light microscopy (including immunofluorescense capable of detecting C3 deposits) and electron microscopy. These analyses did not reveal C3 deposits. The referee is right to point out that lack of C3 deposits makes complement dysregulation a less likely explanation for the pathogenesis of aHUS, yet we have tried to look up in the literature whether C3 depositions are always found in aHUS; the information is not always available and although we agree that the lack of C3 deposition is strange we are not sure if this excludes complement dysregulation as a contributing factor to the disease.
The question whether the G261D mutation has functional effect was previously commented on (referee 4, major comment 1).

We did not make changes in the manuscript due to this comment.

- Referee 4, minor comment 1:
Please improve quality and size of Figure 1

Authors’ response:
We agree with the reviewer that the quality and size of the initially submitted Figure 1 was insufficient and we apologize for the inconvenience. However, it was already noted by the Editor, Dr Henderson, upon submission and we have subsequently uploaded Figure 1 in improved quality.
This improved quality Figure 1 is attached to the current submission, including legend marks as outlined in the response to referee 1. Otherwise no changes have been made.