Author's response to reviews

Title: Chronic kidney disease, severe renal vascular involvement and kidney neoplasia: on the spectrum of kidney involvement in MELAS syndrome.

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Author's response to reviews: see over
To the Editorial Office of BMC Nephrology,

We are very glad that our paper entitled:“Chronic kidney disease, severe renal vascular involvement and two distinct kidney neoplasias: on the spectrum of kidney involvement in MELAS syndrome” was considered as of potential interest for your readership.

In the following lines we have tried to answer the questions and issues of both the Editorial office and the reviewers.

Thus, we would like to thank the editors and the reviewers for the suggestions aimed at improving the quality of our work.

Point by point answers:

Editorial requests:

-Competing interests

Please include a 'Competing interests' section between the Conclusions and Authors' contributions. If there are none to declare, please write 'The authors declare that they have no competing interests'.

Answer: Ok it was done.

Authors' contributions
Please include an Authors' contributions section before the Acknowledgements and Reference list.

For the Authors' contributions we suggest the following kind of format (please use initials to refer to each author's contribution): AB carried out the molecular genetic studies, participated in the sequence alignment and drafted the manuscript. JY carried out the immunoassays. MT participated in the sequence alignment. ES participated in the design of the study and performed the statistical analysis. FG conceived the study, and participated in its design and coordination. All authors read and approved the final manuscript.

Answer: Ok it was done.

**Reviewer's report**

Reviewer: Michael Hughson

Reviewer's report:

The authors describe a patient with MELAS syndrome due to the common 3243 A>G mitochondrial mutation who developed severe renal impairment and nephrotic syndrome following unilateral nephrectomy. I believe the article meets the journal requirements for a case report as it presents new findings in an uncommon genetic disease, and the findings have implications for the development of sclerosing renal disease in the general population. I have questions regarding the pathologic findings and their description and suggest moderately extensive revision.

Some very vague terms are used for the pathology of the kidney that should be changed to more specific pathologic diagnoses or processes.

A. the terms vascular involvement and renal vascular damage are used.

This could mean any type of vascular pathology. The authors illustrate intimal fibrosis of arcuate and interlobular arteries with a wedge–shaped area of interstitial fibrosis and tubular atrophy that appears to extend to the renal capsule. The vascular
pathology should be referred to as intimal fibrosis and the glomerular changes as glomerular obsolescence with the glomerular and vascular changes being of the type associated with benign arteriolonephrosclerosis. It might be mentioned that while arteriolonephrosclerosis is characteristic of benign hypertension, it is often seen in elderly individuals who have never been known to be hypertensive. It can also be noted that some people working in the field (Richard Tracy among others) suggest that the vascular changes precede hypertension.

Answer: We have changed the description of lesions according to the referee’s recommendations, in the abstract, and in the text.

Abstract:

The morphological examination revealed a widespread interstitial fibrosis with dense inflammatory infiltrate and tubular atrophy, mostly with thyroidization pattern. Vascular lesions were prominent: large vessels displayed marked intimal fibrosis and arterioles had hyaline deposits typical of hyaline arteriolosclerosis. These severe vascular lesions explained the different glomerular alterations including ischemic and obsolescent glomeruli, as is commonly observed in the so-called “benign” arteriolonephrosclerosis. Some rare glomeruli showed focal segmental glomerulosclerosis; as the patient subsequently developed nephrotic syndrome, these lesions suggest that silent ischemic changes may result in the development of focal segmental glomerulosclerosis secondary to nephron loss, at least in some cases of MELAS-related nephropathy. Thus the incidence of kidney disease in the “survivors” of MELAS syndrome may increase as the support therapy of these patients improves.
b. Figure 4 shows a hyaline arteriolosclerosis involving two arterioles. This should be noted in the description of the pathology and should be referred to as hyaline arteriolosclerosis. The illustration of focal segmental glomerulosclerosis (FSGS) is not very convincing. A better illustration is needed if it can be provided.

Answer: Indeed the referee is right in the sense that what is observed are the severe vascular changes which anticipated proteinuria in our patient; thus that the full blown picture of FSGS was probably not present but going to develop over time, when a critical amount of the renal tissue was removed at nephrectomy.

We tried to better clarify this point both in the abstract and in the case report:

Morphological examination revealed a widespread interstitial fibrosis and marked vascular changes characterized by intimal fibrosis of arcuate and interlobular arteries with a wedge-shaped area of interstitial fibrosis and tubular atrophy extending to the renal capsule suggestive of ischemic damage (figure 3). Hyaline arteriolosclerosis (figures 3-4) and signs of chronic inflammation were also seen. These severe vascular lesions were the basis of the different glomerular alterations including ischemic and obsolescent glomeruli. The obsolescent glomeruli amounted to approximately 50% of the more than 100 glomeruli sampled. Some rare glomeruli (about 5-10%) showed focal segmental glomerulosclerosis, which in this context can be interpreted as consequent to the vascular alterations.

As the patient subsequently developed nephrotic syndrome, these lesions suggest that silent ischemic changes may result in the development of focal segmental
glomerulosclerosis secondary to nephron loss, at least in some cases of MELAS-related nephropathy.

2. I do not believe that the findings warrant the conclusion that there are twoneoplasms. An oncocyto ma is clearly described and illustrated. What is being diagnosed as a chromophobe carcinoma consists of a 0.5 cm lesion. I doubt that any lesion that small could be considered a chromophobe carcinoma. Unless monosomies of multiple chromosomes characteristic of chromophobe carcinoma could be demonstrated, it would probably be best to consider the lesion a tumoral focus of tubular oncocyto sis and a precursor of another oncocyto ma.

Answer: we rediscussed this issue with our pathologist, and we would like to thank you for the remark and the text was corrected as follows:

Title: CHRONIC KIDNEY DISEASE, SEVERE RENAL VASCULAR INVOLVEMENT AND KIDNEY NEOPLASIA: ON THE SPECTRUM OF KIDNEY INVOLVEMENT IN MELAS SYNDROME.

Text:

Therefore, it is plausible that the mitochondrial DNA mutation detected in our patient was responsible for the development of the closely related cancers (43-44). Moreover, oncocyto ma may be the result of the intrinsic mitochondrial mutation combined with the tendency for neoplasms, including oncocyto mas, to develop in chronically diseased kidneys.
Legend: Figure 2: The smaller lesion (0.5 cm): the figure shows a solid growth pattern and perinuclear cytoplasmic clearing (a). In the box: The tumor contains a population of large, round to polygonal cells with well-defined cell borders and amphophilic, pale basophilic to foamy cytoplasm; nuclei are typically hyperchromatic, elongated and grooved with an irregular nuclear membrane. These features suggest chromophobe renal cell carcinoma; alternatively the lesion may represent a focus of tubular oncycytosis and a precursor of another oncycytoma; the two types of lesions are in any case strictly correlated (43-44) (b) (Hematoxylin eosin stain).

Background: We report the case of a patient with MELAS syndrome and the “classic” 3243A>G mutation of mitochondrial DNA, who developed kidney cancer and severe and rapid kidney functional impairment after unilateral nephrectomy. Analysis of the kidney tissue at a distance from the cancer lesion, sampled in an early phase of kidney disease

Case presentation: In November 2009 the patient underwent right nephrectomy. At surgery, a second small superficial tumor was detected. The histological examination identified a renal oncycytoma (2 cm); a smaller lesion (0.5 cm) had an appearance highly suggestive of chromophobe carcinoma. However, the lesion could also represent a tumoral focus of tubular oncycytosis and a precursor of another oncycytoma (figures 1-2). The cytogenetic analysis was not performed, in this case; however, the two lesions are highly correlated, and a further definition was considered as of minor clinical relevance.
3. It is mentioned that kidney involvement is "protean". I do not know what this means. Most of the reports on the kidney disease of MELAS syndrome describe FSGS.

Answer: both glomerular and tubular damage, resulting in hyponatremia and acquired De Toni Fanconi Debré syndrome are described; furthermore, in several cases, the disease results in end stage kidney disease without specific characteristics (references 13-19). However, we agree with the referee that FSGS is the best characterised lesions. Therefore, this point was clarified in the text, as follows:

Kidney involvement is protean, and both glomerular disease (focal segmental glomerulosclerosis- FSGS) and tubular lesions, resulting in hyponatremia or in a full blown De Toni Fanconi Debré syndrome have been described. Furthermore, in several of the reported cases, kidney involvement has been diagnosed only when advanced renal failure occurred (13-19). The best characterized lesion, leading to kidney biopsy in nephrotic syndrome, is FSGS.

And, in the conclusions:

Reported cases include: glomerular diseases, mainly FSGS; tubular disorders, either with Fanconi syndrome or in the form of salt-losing nephropathies; and end-stage renal failure (2-4, 10-19, 21, 23-24).

4. Under conclusions:

a. Second paragraph. The unusual feature of this case is the severe
arteriolonephrosclerosis occurring without hypertension and the deterioration in renal function and development of nephrotic range proteinuria shortly after nephrectomy for a renal tumor.

Answer,

we agree with the referee that this is an important point; the sentence was therefore modified as follows:

The feature of this case is the severity and complexity of kidney involvement, characterized by kidney cancer and by severe arteriolonephrosclerosis occurring in the absence of hypertension, that we speculate may be the ground for the severe and progressive nephropathy (with the appearance of nephrotic proteinuria, previously absent) which developed shortly after nephrectomy for neoplasia (figures 1-4).

b. The authors refer to steroid resistant FSGS and later to a lack of steroid sensitivity in FSGS. FSGS is virtually always steroid resistant. Do the authors mean steroid sensitive and resistant nephrotic syndrome?

Answer: We do not completely agree with the referee, who is right in stating that the response to steroids is lower than in other forms of nephrotic syndrome, namely minimal change disease. However, steroids are still considered the main first line therapy for FSGS by several authors, and a variable percentage of the patients (30-80%) are reported as steroid responsive. Thus, for example in the third edition of Comprehensive Clinical Nephrology (Feehally, Floege, Johnson, Elsevier 2007), the treatment flowchart for FSGS patients with symptomatic nephrotic syndrome starts with prednisone (1 mg/kg/day for 6-8 weeks, with subsequent taper until remission or
up to 6 months), in addition to BP control, ACEi, statins and avoidance of high protein diet (ibidem page 226).

5. The parallel with 5/6th nephrectomy is well taken. As the authors state, the report seems to be an example of a unilateral nephrectomy associated nephritic syndrome owing to hyperperfusion occurring on a background of severe arteriolonephrosclerosis. The oncocytoma may be the result of the intrinsic mitochondrial mutation combined with the tendency for neoplasms, including oncoeytomases, to develop in chronically diseased kidneys. The title of the paper needs to be changed to reflect the comments.

Answer: thanks for your comment, which was reported in the paper.

The title was changed into:

**CHRONIC KIDNEY DISEASE, SEVERE RENAL VASCULAR INVOLVEMENT AND KIDNEY NEOPLASIA: ON THE SPECTRUM OF KIDNEY INVOLVEMENT IN MELAS SYNDROME.**

In the end, we would like to thank the reviewer for the comments and the suggestions that improved the quality of our study.
Reviewer 2.
Reviewer: Lucia Del Vecchio

Thanks for the comments which improved the quality of our study. In the following lines, we tried to answer to the issues and questions.

Reviewer's report:
The case report is interesting, since little is known about kidney involvement in MELAS syndrome.
I suggest the following clarifications:

1) please discuss that given that histological picture was taken before proteinuria development it is possible, (even if unlikely) that another glomerulonephritis occurred

Answer: thanks for raising this issue:
The sentence “before the appearance of proteinuria” was added in the abstract and again in the presentation of the case, and in the conclusion, as follows:

The case: Of note, the changes were recorded before the onset of proteinuria and in the absence of hypertension, both at nephrectomy and over the follow-up.

The conclusion:
... and by the severe arteriolonephrosclerosis occurring without hypertension, that may be the ground for the severe and progressive nephropathy (with nephrotic proteinuria, previously absent) which developed shortly after nephrectomy for neoplasia (figures 1-4).
And again:

**Our patient was normotensive and non-proteinuric at the time of nephrectomy, even if his renal function had probably started to decrease.**

The issue of a different association was mentioned as follows:

**Our patient was normotensive and non-proteinuric at the time of nephrectomy, even if his renal function had probably started to decrease.**

**The referee asked:**

2) electron microscopy analysis was not made. This should be written as a possible limitation of this case report (no data about podocytes status).

**Answer:**

This is an interesting remark, this point was added as follows:

unfortunately electron microscopy was not available in our case, thus impairing any conclusion on podocyte status in our patient

3) please give a better description of the histological picture in the text. How many glomeruli were analysed? in which percentage had they global sclerosis? In which percentage was focal segmental sclerosis? in the end, had the patient enough criteria to fulfill a histological diagnosis of focal segmental glomerulosclerosis?

These points were clarified in the text, as follows.

**Morphological examination revealed a widespread interstitial fibrosis and marked vascular changes characterized by intimal fibrosis of arcuate and interlobular arteries with a wedge-shaped area of interstitial fibrosis and tubular atrophy extending to the renal capsule suggestive of ischemic damage (figure 3).** Hyaline arteriolarosclerosis
(figure 3-4) and signs of chronic inflammation were also seen. These important vascular damages are the basis of the different glomerular alterations including ischemic and obsolescent glomeruli. Some rare glomeruli showed focal segmental glomerulosclerosis, which in this context can be interpreted as the consequence of vascular alterations.

As the patient subsequently developed nephrotic syndrome, these lesions suggest that, at least in some cases of MELAS-related nephropathy, silent ischemic changes may result in the development of focal segmental glomerulosclerosis secondary to nephron loss.

4) please specify that the patient developed type 2 diabetes.

The definition of diabetes in MELAS syndrome is not so clear-cut, and probably mitochondrial diabetes is presently the most frequently used. Thus, we preferred to refer to “diabetes mellitus” or “diabetes”, following the choice of most of the recent literature reports. However, we agree that this is an important point and the problem of definition of the type of diabetes was specified as follows, in the conclusion paragraph:

defined by different Authors as type 2 diabetes, or as mitochondrial diabetes.

Thanks again for the help and for the interesting suggestions.

Hoping that we have answered all the previous questions,

sincerely yours,

Giorgina B Piccoli, and co-authors