Author’s response to reviews

Title: Case Report: Extreme coronary calcifications and hypomagnesemia in a patient with a 17q12 deletion involving HNF1B

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Dear Editors:

We thank you and the Reviewers for the excellent comments and suggestions for improving our manuscript. In response, we have made revisions according to the comments of the Reviewers. Below is a point-by-point enumeration of the Reviewers’ comments (in bold text), along with our responses and the corresponding revisions to the manuscript where appropriate (in italics with yellow highlights to distinguish new material within existing sentences). The references cited in our responses to the Reviewers’ comments are numbered according to their appearance within this letter (see references at end of this letter) but are numbered differently in the actual text of the manuscript. We have addressed each and every point raised by the Reviewers, and we believe that this process has resulted in a substantially improved report of our work. We would be happy to make any further revisions you or the Reviewers deem necessary.
Pasquale Mario Fatuzzo (Reviewer 1):

1. This is an interesting and well written case report on new vascular effects of 17q12 deletion. The detection of important coronary artery calcifications and the possible mechanisms at the basis of this process have been well reported for the first time. Few questions, at discretion of the authors, could be answered.

We thank the Dr. Fatuzzo for his supportive comment.

2. Authors linked the vascular effect of 17q12 deletion to CKD-MBD. Were calcifications limited to coronary arteries or involved other districts (i.e., aorta)?

Yes, while the high degree of calcification in this patient’s coronary arteries was most striking, whole body CT imaging also noted arterial calcifications in the cerebral and carotid arteries. Descending aorta and iliac arteries demonstrated a few calcific spots, and no calcifications were noted in the renal or mesenteric arteries. We have added these details to the case description (Case Presentation section, page 4):

- CT scan showed vascular calcifications of the anterior and posterior cerebral arteries, bilateral carotid arteries, and calcific spots along the descending aorta and iliac arteries. Notably, there was extensive calcification of the coronary arteries (Figure 1), with a coronary artery calcium (CAC) score of 12,427 by the Agatston scoring method [1].

3. Is it possible to check whether arterial calcifications involved intima or media layer? Have any data on elastic and muscular artery stiffness (i.e., carotid-femoral and carotid-radial pulse wave velocity)? Is it possible for the authors to perform a non-invasive study of functional and structural arterial wall properties (i.e., at the level of the carotid artery, using an echotracking device)?

We agree with Dr. Fatuzza that these studies would have yield interesting insights into the nature of our patient’s calcifications and their underlying pathophysiology. Patients seen through the NIH Undiagnosed Diseases Program are brought to the NIH Clinical Center for evaluation and clinical phenotyping. Both invasive and noninvasive studies are performed based on a discussion between patients, staff clinicians, and investigators weighing the costs, risks, and benefits of such studies. Unfortunately, arterial biopsies, shear wave elastography, and other advanced imaging beyond what is described in the case report were unable to be performed before the patient was discharged to the community.
Gema Ariceta (Reviewer 2):

1. Your manuscript entitled "Case Report: Extreme coronary calcifications and hypomagnesemia in a patient with a 17q12 deletion involving HNF1B, describes a patient with hypomagnesemia of renal origin and bilateral renal cysts associated with MODY type diabetes, and severe coronary artery calcification, who was discovered to be caused by 17q12 deletion involving HNF1b gene and other 15 genes. The patient was diagnosed at 62 years of age, and presented with neurologic symptoms as well. The case report is well described and is of interest, and further, demonstrates that even in senior patients monogenic diseases can be discovered. In this patient, systemic involvement of different systems could raise an alert of a unique cause, whereas a large 17q12 deletion could explain the complex phenotype and mainly the neurologic picture. I support this manuscript for publication, however before that, I recommend some changes and comments, detailed below.

We thank the Dr. Ariceta for her supportive comments.

2. Lines 87-90 authors missed to comment about the presence of hypokalemia and hypocalcemia at baseline. Further, it would be important to remark if the presence of hypercalciuria was observed before starting treatment with furosemide, of it could be secondary to diuretic use. That concept is key as disorders at the ascending thick segment of loop of Henle cause hypermagnesuria and hypercalciuria, whereas disorders at the distal convoluted tubule cause loss of magnesium in urine and not increase loss of calcium in urine.

Our intention in discussing these laboratory findings in Lines 87-90 was to refer laboratory evaluation at the NIH in January 2018, during which hypokalemia and hypocalcemia were no longer noted. The patient’s historical hypokalemia and hypocalcemia are discussed earlier in Lines 65-68. We apologize for this confusion and have edited our wording to make this more clear (Case Presentation, page 4, paragraph 3):

- Laboratory evaluation at the NIH in January 2018 was significant for hyperparathyroidism and increased urinary magnesium excretion with an inappropriately high fractional excretion of magnesium of 23% based on 24-hour urine collection (normal < 4%), and slightly increased urinary calcium excretion. Serum electrolytes were normal while receiving oral and intravenous magnesium repletion at the time of NIH evaluation. Aside from magnesium, urine electrolytes were normal (Table 1).

We also thank Dr. Ariceta for her important point about the potential role of diuretic exposure to urinary magnesium and calcium excretion. We have clarified the details of our patient’s history of diurectic exposure in the case history (Case Presentation, page 4, paragraph 3).
• One week after starting triamterene-hydrochlorothiazide for hypertension, he complained of profound malaise, myalgia, and arthralgia and was found to have severe electrolyte abnormalities: hypomagnesemia, hypocalcemia, and hypokalemia (Table 1). Aside from his one week trial of triamterene-hydrochlorothiazide, the patient had not taken any other diuretics.

3. Family history: could other members of the family be tested for 17q12del? Authors said there were not history of consanguinity. Please state that HNF1beta deletions are very frequently "de novo mutations" and they are inherited as dominant trait,… therefore lack of consanguinity had not a major impact on the diagnosis suspicion

We thank Dr. Ariceta for this comment, and have appended our discussion to clarify this important point (Discussion, Page 5, Paragraph 2):

• While the inheritance of these renal syndromes have been described as autosomal dominant traits, deleterious HNF1B polymorphisms frequently arise from de novo mutations.

Discussion

4. Please add that other causes of urinary calcium and magnesium loss could be related to CaSR and lack of action of HNF1b on claudin 19 and 14 among others

We have edited our discussion to include a more thorough discussion of genetic causes of hypomagnesemia (Discussion, Page 6, Paragraph 3).

• Increased renal excretion can be due to excessive diuresis, medications (particularly loop and thiazide diuretics), acquired tubular dysfunction, and more rarely, genetic causes such as Gitelman and Bartter syndromes and defects of tubular components such as CaSR, claudins 19 and 14, among others.

5. Lines 156-160. The explanation that permanent Mg wasting could be attributed to subclinical acute nephrotoxic episodes and parallelism with cisplatin toxicity is not convincing in this patient. Please introduce other ideas at the discussion such as:

i) Hypomagnesemia linked to HNF1b mutation has been described to very more frequent and severe over time , with age, and thus, it is expected in a 62 y, old patient

ii) HNF1b deletion causes more severe phenotype, and this patient had a large deletion involving 15 genes,... could be the clinical picture the resulting effect of other gene?
Discus the role of CKD in this patient with bilateral cysts,... and chronic tubulointerstitial disorder too. Consider the potential consequences of chronic untreated hypokalemia on cysts formation, CKD and tubulointerstitial disease.

Was patient diabetes well controlled? Often hypoMg is associated with bad controlled diabetes.

We thank Dr. Ariceta for this suggestion to improve our discussion. We have expanded on our discussion of late-onset, irreversible Mg wasting by discussing age-related worsening of disease presentation, the extent of the patient’s genetic deletion, and the presence of metabolic and cardiovascular comorbidities and structural renal disease (Discussion, Page 7, Paragraph 2):

- Additionally, manifestations of HNF1B haploinsufficiency may worsen with age, as both childhood and late-adulthood presentations of HNF1B-related disease have been described [2]. Variability in HNF1B-related hypomagnesemia may be influenced by the extent of 17q12 deletion (1.5 megabases involving 15 genes in this patient), the effect of metabolic and cardiovascular comorbidities (obesity, hypertension, and diabetes), and the presence of structural renal disease (renal cysts).

6. Associated family cardiovascular risk factors: did other family members have a genetic diagnosis?

Family history of cardiovascular disease (sudden cardiac death, aortic aneurysm) are discussed in the Case Presentation, Page 4, paragraph 2).

We have clarified that genetic counseling and testing was offered to family members, but family members declined genetic diagnosis at the time of evaluation (Case Presentation, Page 5, Paragraph1):

- Genetic counseling was offered, and family members did not elect for genetic testing at the time of evaluation.

7. Hypomagnesemia has been observed to produce HTN, could be another CV factor in this patient?

We thank Dr. Ariceta for bringing this excellent point to our attention. We have added this point to our discussion regarding the etiological link between hypemagnesemia and vascular calcification in this patient (Discussion, Page 10, Paragraph 1):

- Finally, at the systemic level, there is some evidence that hypomagnesemia itself may exacerbate hypertensive disease, a critical risk factor for atherosclerosis [3, 4].
8. Please make a comment on patient treatment. How could you justify the continuous use of furosemide? Could that diuretic be the cause of moderate hypercalciuria and worsening urinary magnesium loss?

We are happy to clarify this point. The patient was not taking furosemide at the time of initial presentation. The patient’s diuretic use at time of presentation is described in Case Presentation, Page 3, Paragraph 3:

- Aside from his one week trial of triamterene-hydrochlorothiazide, the patient had not taken any other diuretics.

Following the patient’s initial presentation (but prior to evaluation at our institution), the patient was started on furosemide 40 qd for hypertension management by his community provider. At that time, it was noted that the addition of furosemide improved his hypertension control without affecting his magnesium supplementation requirements. While furosemide and other diuretics may certainly exacerbate urinary magnesium loss, close monitoring of this patient’s electrolytes revealed that this effect was not found to be significant in this patient, and the benefits outweighed the risks.

References


