Dear editor,

We would like to thank you and the reviewers for careful reading of our manuscript. We have given the comments serious consideration and altered the manuscript according to the suggestions. Most importantly; 1) we tested additional tissue from previous operations of colon, spleen and nasal polyps in which the RET mutation was confirmed; making mosaicism less likely, 2) we excluded additional mutation in the RET gene and 3) calculated the chance of de novo by Bayesian statistics and clarified the pedigree (figure 1). Below you will find a point-by-point response to all of the issues raised in the reviews. In the attachment you will find the manuscript marked with our changes.

Due to the additional analyses and revision of the manuscript we would like to add Tom van Wezel as a co-author.

We hope that the revised manuscript will meet your expectations and we are willing to answer any other questions you might have.

Yours sincerely,

Karin van der Tuin, on behalf of all the authors.

Reviewer 1: Major revision

1. The possibility of mosaicism is considered in the manuscript, and ultimately dismissed as unlikely, in part on the basis of the presence of the mutation in both the blood and the germline. Given that no other tissues have been examined (and in particular the thyroid was not tested, and neither was the prostate although the patient had prostate cancer, so tissue was possibly available), mosaicism cannot be formally excluded, and remains a very plausible explanation for the lack of phenotype. I recommend that the manuscript be revised in this spirit. The phrase claiming that in cases of mosaicism the mutation is absent from the peripheral blood (page 7, “Alternatively, ...”) is not scientifically correct (the mutation might be present in the blood, the blood itself might be mosaic) and should be deleted.

A: In addition we tested tissue from previous operations of colon, spleen and nasal polyps in which the RET mutation was confirmed; making mosaicism less likely.

2. The manuscript proposed that genetic modifiers may be responsible for the incomplete penetrance of the mutation (assuming absence of mosaicism). Since rare scenarios are considered to explain this very rare case, one should go all the way: There is the possibility that this modifier resides on the actual mutant RET allele of the elderly carrier, cancelling out the effect of the mutation (for example, a nonsense mutation), and was removed by genetic recombination in the germline, thus passing only the pathogenic mutation to the affected son. This theoretical possibility should be excluded by sequencing the whole coding sequence of the RET gene in the 93yo carrier (and, if sequence variants are found, they should be screened for in the affected son).
A: No additional pathogenic mutations were found during sequencing of the whole coding sequence of the RET gene.

3. It is very unfortunate that a thyroid ultrasound was not performed in the 93yo carrier (nor apparently a calcitonin measurement). Is there any imaging available from the prostate cancer staging that might include the thyroid (e.g., a CT scan)?

A: No clinical or laboratory diagnostic evaluation was performed during hospitalisation that would have indicated the presence of MTC, pheochromocytoma or primary hyperparathyroidism (no Ctn, calcium, catecholamine or PTH levels and no thyroid ultrasound or CT-scan of the head neck region). No autopsy was conducted.

4. There is a discrepancy between the text and the Table regarding the age of recommended prophylactic thyroidectomy in carriers of a RET codon 620 mutation: before age 5 years according to the text (page 7, second paragraph) vs. childhood or young adulthood according to the Table. Which one is correct? The discrepancy should be corrected.

A: The recommendation of prophylactic thyroidectomy is during childhood or young adulthood, we changed that in the text.

Reviewer 2: Minor revision

General comments:

In this case report, van der Tuin and coworkers presented a 93-year old MEN2A mutation carrier without symptoms of medullary thyroid carcinoma (MTC). However, the authors report that the carrier denied any further clinical evaluation for MEN2A. Although, MTC would be probably apparent by the age of 93, it is interesting to know if the carrier underwent at least ultrasound of neck in order to exclude MTC. In addition, a mild hyperparathyroidism might not be clinically obvious. The case is interesting but authors should give some additional information for the evaluation of this carrier in order to draw definite conclusions.

Specific comments:

Since the carrier diagnosed also with metastatic prostate carcinoma, did he have any clinical or laboratory diagnostic evaluation that could elucidate his MEN2A status (calcitonin levels, calcium levels, intact PTH levels, CT-scan of neck / abdomen)? Even if not, the authors may consider to expand little more concerning the evaluation of the carrier. There only 3,5 lines of text referred to him.

A: We added more clinical information about the 93-year-old mutation carrier. “No clinical or laboratory diagnostic evaluation was performed during hospitalisation that would have indicated the presence of MTC, pheochromocytoma or primary hyperparathyroidism (no Ctn, calcium, catecholamine or PTH levels and no thyroid ultrasound or CT-scan of the head neck region). No autopsy was conducted.”
The authors should start the case presentation with the 93 year old father and then describe the clinical data for his 50y old son.

**A: The 50-year-old son is the proband in this family, therefore we would like to describe him first.**

The authors stated that no other parental family members were identified as mutation carries. How this is justified since there is a second generation member that was not available for DNA test and this member was offspring of one of the non tested first generation member?

**A: Bayesian statistics lower the a prior chance of inherited disease from 95% to a post prior chance of 0.05%. Calculation (in short): A prior chance inherited RET mutation is 95%. Odds = 95 x (1/2)¹¹ x (3/4)¹⁰ = 0.0026. Post prior chance 0.052%.**

Table I. : A883F is a high risk (H, ATA group) mutation and not HST.

**A: We changed that in the table.**

**Reviewer 3: Minor revision**

An interesting and well-written manuscript with a case supporting the theory of lower MTC penetrance in C620R carriers than otherwise thought.

page 1, line 4

The title of the manuscript is “A 93-year-old MEN2A mutation carrier without Medullary Thyroid Carcinoma: a case report and review of the literature”. However there is no flow chart or method section explaining the literature review. I propose either to omit the term “literature review” or do a systematic review, which will of course heighten the quality of the manuscript.

**A: We changed the term literature ‘review’ in ‘overview’.**

p. 3, lines 7-8 and page 5, line 3, page 8, line 16

I agree that the mutation of the proband’s father is very likely to be de novo. However since there is no RET screening results available for the parents of the proband’s father (the 93-year-old) it seems reasonable to change “de novo” to “apparent de novo”.

**A: We changed “de novo” to “apparent de novo”.**

page 5, line 11

Please mention whether the patient pre-operatively was screened for PHEO due to the elevated levels of serum Ctn and CEA.

**A: Added to the manuscript; “No pheochromocytoma screening was performed at that point of time.”**

page 5, line 5
Please mention whether the family showed any signs of HD since HD is prevalent in 50% of MEN2A patients with mutations in codon 620 (http://www.ncbi.nlm.nih.gov/pubmed/25810047).

A: Added to the manuscript; “The family history showed no signs of Hirschsprung’s Disease.”

page 5, line 24

Please state that the father of the proband never had any thyroid surgery if this was the case.

A: Added to the manuscript; “The father of the proband was also identified as a carrier of the RET-mutation at an age of 91 years old without thyroid surgery or any symptoms of the thyroid or other health problems.”

page 8, line 4

A recent reference for interfamilial phenotypic variability could also be (http://www.ncbi.nlm.nih.gov/pubmed/25515555).

A: Thank you for mentioning this really interesting article. We will not include this article because it is specific for exon 11 mutation whether in our family an exon 12 mutation is present.

page 12, line 16

Please correct that the A883F mutation is no longer in the ATA-HST category but has been placed in the ATA-H (http://www.ncbi.nlm.nih.gov/pubmed/25810047).

A: We changed that in the table.

Reviewer 4: Minor revision

The Authors describe here a case of a 93-year-old male carrier of a MEN2A mutation, who did not develop during his life span neither a Medullary Thyroid Carcinoma nor other features of the syndrome.

Apparently, the subject – father of the proband son affected by the complete syndrome – died for a respiratory insufficiency and an advanced prostatic carcinoma. Unfortunately, autopsy was not performed, no neck ultrasounds, no CT levels, no any material was available to complete the diagnosis a part the blood sample on which the genetic analysis was performed. This is the only black hole in the description of the case. However, he was not referred for any clinical symptom related to a MEN2A syndrome.

The family pedigree and the clinical features of affected individuals are described correctly.

Review of the literature is updated and exhaustive.

The possible causes of the absence of a clinical manifestation of the syndrome are analyzed and discussed; particularly various genetic modifiers are mentioned.
Given the fact that prophylactic thyroidectomy is usually offered to carrier of the mutation and however intensive screening is performed, the possibility of not developing the disease is surely relevant. Even in a single case. Moreover, the subject lived 93 years, therefore had all the time to develop tumorigenesis. We know that in the cases with manifest disease this is very aggressive and at a very young age. The son of the proband (19-year-old) had already 3 foci of MTC at histology. We have observed the same condition in even younger patients.

In conclusion, the case described by the Authors is worth the publication. I would add few words of considerations concerning the fact that no other material was available to exclude the clinical diagnosis.

A: We added more clinical information about the 93-year-old mutation carrier. In addition, we tested tissue from previous operations of colon, spleen and nasal polyps in which the RET mutation was confirmed; making mosaicism less likely.

Correct on page 7, 2nd paragraph, before citation 9: “permanent hypoparathyroidism”, not hyper.

A: We corrected “hyperparathyroidism” to “hypoparathyroidism”.

(END)