Title: Identification of a novel PAX8 gene sequence variant in four members of the same family: from congenital hypothyroidism with thyroid hypoplasia to mild subclinical hypothyroidism

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Author’s response to reviews:

June 3rd, 2014

Professor Timothy Shipley,
Executive Editor, BMC Endocrine Disorders;
Dr Amar Agha
Editorial Board, BMC Endocrine Disorders;

Re: MS No. 6888624791205151

Dear Dr. Agha,

Thank you for kindly considering the paper we submitted, a revised version of which is herewith enclosed. We have incorporated most of the reviewers’ suggestions into this revised version, and we will provide a point-by-point reply to those suggestions.

Reviewer 1, Dr Edna Roche:

We thank the Reviewer for evaluating our manuscript with care and attention and for providing us with valuable suggestions.

Major compulsory revisions:
1. We modified the Introduction in line with your suggestion, as follows: “The aim of this report is to analyse PAX8 gene in members of the same family with variable phenotypic expressivity: from congenital hypothyroidism with thyroid hypoplasia, to mild subclinical hypothyroidism, in order to establish if a correlation between variants in the PAX8 gene and different phenotypes is present.” This point is mentioned again in the abstract: “The aim of this study was to analyse the PAX8 gene sequence in several members of the same family in order to understand if the variable phenotypic expression ranging from congenital hypothyroidism with thyroid hypoplasia to mild subclinical hypothyroidism is associated to a genetic variant in the PAX8 gene.”

2. We agree that in the methods section it is not described how the control groups were selected but we want underline that this study is not a case control study. Nevertheless, we modified the Methods as follows: “In order to exclude the occurrence of the new PAX8 genetic variant as a common polymorphism, 115 healthy Caucasian European subjects have been screened for the same substitution. They signed written informed consent for the genetic analysis. In addition, 26 patients with congenital hypothyroidism followed at Pediatric Endocrinology Division of Verona Hospital during 2011, were screened for the same substitution after their parents’ written consent. All of them were older than fourteen and were in L-thyroxine treatment from birth. Thirty-one per cent of them did not present echographic alterations, 25% showed thyroid hypoplasia, 31% ectopia and the remaining 13% agenesis of the gland”. Moreover we modified the Results: “The R133W variant was not present in a screen of 115 Caucasian European subjects (230 control chromosomes), thus reducing the likelihood that this mutation represents neutral common variant. In addition, 26 hypothyroid children (52 control chromosomes) were analysed and none carried the R133W variant”.

3. We reviewed the conclusions, in accordance with your suggestion. We modified the conclusions as follows: “Although in vitro data do not prove that R133W-PAX8 is directly involved in the development of the thyroid phenotypes reported for the carrier’s family members, it is reasonable to conceive that, in case of transcription factors such as Pax8, which establishes several interactions in different protein complexes, genetic variants could have an impact in vivo, that still has to be thoroughly investigated. Probably, genetic, epigenetic and environmental factors are involved in these pathways and only further studies can confirm our hypothesis”. This point is mentioned again in the abstract: “Although in vitro data do not prove that R133W-PAX8 is directly involved in the development of the thyroid phenotypes reported for the carrier’s family members, it is reasonable to conceive that, in case of transcription factors such as Pax8, which establishes several interactions in different protein complexes, genetic variants could have an impact in vivo, that still has to be thoroughly investigated”.

4. We amended the abstract according to your comments, as described above.

Minor essential revisions:

5. We modified the manuscript according to all your suggestions.
Reviewer 2, Dr Seamus Sreenan:

We thank the Reviewer for evaluating our manuscript with care and attention and for providing us with valuable suggestions.

Discretionary revisions:

1. We clarified the aim of the study, as you requested, in the abstract as follows: “The aim of this study was to analyse the PAX8 gene sequence in several members of the same family in order to understand if the variable phenotypic expression ranging from congenital hypothyroidism with thyroid hypoplasia to mild subclinical hypothyroidism is associated to a genetic variant in the PAX8 gene”. And in the Methods section of the abstract we specified: “We screened an hypothyroid child with thyroid hypoplasia for mutations in PAX8, TSHR, NKX2.1, NKX2.5 and FOXE1 genes. We studied the inheritance of a new variant of PAX8 gene in 9 members of the proband’s family, in 115 Caucasian European subjects and in 26 hypothyroid children. Functional studies was performed to assess the in vitro effect of PAX8 gene variant identified.” Other genes (TSHR, FOXE1, NKX2.1, NKX2.5) were here mentioned to clarify that they were screened to exclude other genetic causes of hypothyroidism, but this analysis was not the aim of our study. Moreover, we modified the introduction as follows: “The aim of this report is to analyse PAX8 gene in members of the same family with variable phenotypic expressivity: from congenital hypothyroidism with thyroid hypoplasia, to mild subclinical hypothyroidism, in order to establish if a correlation between variants in the PAX8 gene and different phenotypes is present.”

2. We have added the information you requested to the introduction, as follows: “In previous studies mutations in PAX8 gene have been detected in patients with thyroid dysgenesis; some of them have athyreosis but the major part of subjects have a hypoplastic but normally located thyroid gland, often associated with renal anomalies 2,16-19,21.”

Minor essential revisions:

1. The manuscript has been revised for the grammatical and linguistic errors as you suggested.

2. We corrected our manuscript using PAX8 when referring to the gene and Pax8 to the gene product.

3. We revised our hypothesis according to your comments and thus modified sentences in the discussion section as follows: “It is well known that persistent stimulation by increased plasma TSH levels leads to thyroid proliferation and often nodule formation 31, 32. This clinical observation could account for the similar phenotype of father, daughter and cousin (patients II-3, III-1 and III-3, the first 2 evidencing thyroid nodules), and for the substantial difference from the propositus, patient III-2, who was treated from birth with substitutive therapy and displayed no thyroid nodules, at the moment.”

4. Thank you for your observation. The thyroid blood tests were not always performed in the same laboratory because the family does not live close to the hospital. While the proband was followed by our service and his blood tests were performed in our hospital, the other family members were not always submitted
to blood examination in our hospital. They often preferred a laboratory nearer to their home. Nevertheless we do not consider this a fault because the data of blood tests are merely described and were not submitted to statistical analysis.

Major compulsory revisions:

5. We agree with you that the cousin of the proband presents a very subtle degree of thyroid dysfunction. Unfortunately, he lives in another town, so we have not specific information about his follow up. We know that he made periodical exams of thyroid function and that at the moment he still presents a subclinical hypothyroidism but he takes no therapy. According to your suggestion, we modified the Method section as follows: "His thyroid function was periodically checked in the hospital of the town where he now lives. We know that his subclinical hypothyroidism persisted but at the moment he takes no therapy. No other clinical abnormalities were found for this patients". This point is mentioned again in the discussion: "we cannot assume that the phenotype described in the proband’s family is due to the PAX8 genetic variant: the cousin showed a very mild phenotype, wild type family members with no clinical signs have not been submitted to thyroid ultrasound, as the Caucasian subjects enrolled for the genetic variant screening".

6. Regarding the thyroid function in the other members of the family, in accordance with your suggestion, we added the following sentence to the Methods: "In all the other relatives reported in Figure 1 thyroid function was tested and TSH, fT4 and fT3 values were in the normal range. No relevant clinical alterations were described for them. Obviously, given the family history, we recommended to these unaffected family members a periodic follow-up. At the moment we have not information of alterations in their thyroid function."

7. We modified the Methods in line with your suggestion, as follows: “Abdomen ultrasound showed no abnormalities. His growth, renal function (at blood tests), neuropsychological development and IQ (measured several times and with different test depending on the age) were normal. […] He did not present neurological alterations. Renal function was in the normal range for age. […] No neurological or renal dysfunctions were evidenced. […] No other clinical abnormalities were found for this patients.”

8. We agree that is possible that the genetic variant and the clinical phenotype may be not related and consequently we modified the Discussion as follows: “Naturally, we cannot assume that the phenotype described in the proband’s family is due to the PAX8 genetic variant: the cousin showed a very mild phenotype, wild type family members with no clinical signs have not been submitted to thyroid ultrasound, as the Caucasian subjects enrolled for the genetic variant screening. Nevertheless, we suppose that the R133W variant may be associated to the phenotype of hypothyroid patients of this family, as only the carriers’ family members presented anomalies of thyroid function and alterations of gland structure.”

Reviewer 3, Dr Ciara McDonnell:

We thank the Reviewer for evaluating our manuscript with care and attention and for providing us with valuable suggestions.
Minor essential revisions:
1. We corrected the syntax errors and the spelling mistakes, according to all your suggestions.

Discretionary revisions:
2. Regarding the clinical description of proband and his family, in accordance with your suggestion, we added the following to the Methods: “His growth, renal function (at blood tests), neuropsychological development and QI (measured several times and with different test depending on the age) were normal. […] He did not present neurological alterations. Renal function was in the normal range for age. […] No neurological or renal dysfunctions were evidenced. […] No other clinical abnormalities were found for this patients. […] In all the other relatives reported in Figure 1 thyroid function was tested and TSH, fT4 and fT3 values were in the normal range. No relevant clinical alterations were described for them. Obviously, given the family history, we recommended to these unaffected family members a periodic follow-up. At the moment we have not information of alterations in their thyroid function.”

Yours sincerely,
Paolo Cavarzere

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