Author's response to reviews

Title: Molecular Diagnosis and Comprehensive Treatment of Multiple Endocrine Neoplasia Type 2 in Southeastern Chinese

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Author's response to reviews: see over
Responses to the reviewers’ comments

Dear Ms. Sarah Jane Millare,

Thank you very much for your kind email of 13 December 2014 regarding our paper “Molecular Diagnosis and Comprehensive Treatment of Multiple Endocrine Neoplasia Type 2 in Southeastern Chinese” (MS. ID: 1644361667140129) with the comments from the reviewer. We very much appreciate your comments and suggestions, which were valuable for improving the quality of our manuscript. Accordingly, we have carefully revised the manuscript. All changes have been highlighted in red. Point-by-point responses to the questions/comments raised by the reviewer are listed below.

We hope, with these modifications and improvements based on your suggestions and the reviewers’ comments, the quality of our manuscript would meet the publication standard of the Hereditary Cancer in Clinical Practice.

Thank you and the reviewer again.

Kind Regards,

Xiao-Ping Qi

Jan 4. 2015

Revision list according to the comments from Reviewer

Q1: Hyperparathyroidism (HPT) is said to be absent in these cases. However, the serum values of PTH, Calcium and PTH/calcium rates of each patient are lacking, which prelude further conclusions.

A1: We accept the suggestion and have added the contents in the text (see Page 7,
Q2: values of cathecolamines, metanephrines, CEA, phosphate, etc of each case was not added. They should be shown in a table or in the text.

A2: Thank you for your comment. We have added catecholamine data in table 4, CEA values in table 3, and phosphate data in the text (see Page 7, lines 24-25).

Q3: Results – page 7, line 11 – it would be better to analyze index-cases and screening family members separately.

Page 7, line 19 and in other parts of the ms: try to correlate phenotype data with genotype findings

A3-1: Thanks for your suggestion. We have described index-cases and screening family members separately in the text (see Page 7, line 12-16).

A3-2: We have added correlated phenotype data with genotype findings in the text (see Page 7, line 16-25; Page 8, line 1-2 and Page 10, line 6-7).

Q4: the Discussion should be shortened in at least 30%; avoid repetition.

A4: Thanks for your thoughtful comment. The discussion had shortened in 30% (see Page 11, line 18-Page 14, line 21).

Q5: Please comment on several cases that were submitted to total thyroidectomy at relatively later ages, considering the ages mentioned in the ATA recommendations (Kloos et al 2009). Also, discuss on some cases that were considered cured despite relatively late thyroid surgery.

A5: Thanks for your comment. We have added the relevant content in the discussion.

i) The efficacy of radiotherapy, chemotherapy and $^{131}$I isotopes therapy is very
limit, radical excision is the only current potentially curative therapeutic method for MEN2-MTC. Targeted therapies block the \textit{RET} receptor tyrosine kinase or its multiple downstream pathways are currently being evaluated in multicenter trials to distant metastases. (Kloos \textit{et al} 2009) (Schneider DF and Chen H. New developments in the diagnosis and treatment of thyroid cancer. \textit{CA Cancer J Clin}, 2013;63:373-394.)

ii) The 2009 ATA recommendations prophylactic level VI compartmental dissection unless there is clinical or radiological evidence of lymph node metastases, or thyroid nodules $\geq 5$ mm in size at any age, or a basal serum calcitonin $\geq 40$ ng/L, and MEN2-related MTC with distant metastases found with calcitonin levels almost greater than 400 ng/L. Patients harboring \textit{RET} proto-oncogene mutations who have clinical or radiographic findings suspicious for metastatic MTC, including those with thyroid nodules 5 mm or a serum Ct level $>40$ ng/L, should be considered thyroidectomy and level VI compartmental dissection and at least the metastasis side of lateral neck compartmental dissection of image or biopsy positive compartments (N1+).(Kloos \textit{et al}). In our group, 20 symptomatic patients who found at older age, and submitted to nonstandard thyroidectomy.

The main reason is the patients’ awareness of this disease, systematic family screening only conducted in our hospital in recent years (between 2005 and 2013).

iii) In a population-based study, the 10 years MEN2-MTC disease specific survival exceeded 90% in patients with localized disease, however, it decreased to 78% and 40% respectively in MEN2-MTC patients with regional or distant metastases. Only 10% of MEN2-MTC patients with metastases to cervical nodes are cured by thyroidectomy and extensive lymph node dissection. The post-Ct falls within the
normal range in 60% of MEN2-MTC patients with node negative disease but in only 10% of MEN2-MTC patients with node positive disease. (Wells et al 2013[4])

iv) The prognosis is excellent in MEN2 patients who have a pre-Ct less than 150ng/L and MTC smaller than 1 cm with no lymph node metastases (Machens A, Schneyer U, Holzhausen HJ, Dralle H. Prospects of remission in medullary thyroid carcinoma according to basal calcitonin level. *J Clin Endocrinol Metab* 2005; 90:2029-2034.). The 10 years survival approaches 100% in MEN2-MTC patients with undetectable basal and stimulated calcitonin levels after initial thyroidectomy (Modigliani E, Cohen R, Campos JM, et al. Prognostic factors for survival and for biochemical cure in medullary thyroid carcinoma: results in 899 patients. *The GETC Study Group. Groupe d’etude des tumeurs a calcitonine. Clin Endocrinol (Oxf)* 1998; 48:265-273).

v) Indeed, an elevated calcitonin level is a highly sensitive marker for MTC that can be used for screening, diagnosis, prognostic assessment, and follow-up monitoring.(Costante G, Durante C, Francis Z, et al. Determination of calcitonin levels in C-cell disease: clinical interest and potential pitfalls. *Nat Clin Pract Endocrinol Metab*, 2009;5:35-44.) However, gender-specific basal calcitonin cut-off for the identification of MTC has been defined, and patients with subclinical MTC (minimal residual or recurrent MTC) or their relatives (with C-cell hyperplasia) usually have normal basal calcitonin levels and stimulation tests are necessary to establish the diagnosis. (Vainas I, Marthopoulos A, Chrisoulidou A, et al. Calcitonin stimulation tests for the early diagnosis and follow-up of patients with C cell disease: a descriptive analysis. *Hippokratia*, 2013;17:246-251.) and (Mian C, Perrino M, Colombo C, et al. Refining calcium test for the diagnosis of...
medullary thyroid carcinoma: cut-offs, procedures and safety. *J Clin Endocrinol Metab.* 2014; 99:1656-1664.) At the 9th meeting of the European Thyroid Association and Cancer Research Network (ETA-CRN), there were comments reported to the 2009 ATA guidelines that pointed out that timing of prophylactic TT should be at best synthetically decided by combining the results of both RET gene testing and the ongoing pre-Ct level (Jarzab *et al* 2013 [9]) Elisei *et al.* [8] observed that in 20 RET mutation carriers operated on for detectable basal calcitonin, lymph node metastases were never found at histology in those who had basal calcitonin lower than 60ng/L. and considered that the time of thyroidectomy in RET gene carrier with negative calcitonin could be personalized and safely planned when stimulated calcitonin becomes positive, independent of the type of RET mutation and patient’s age, and a basal calcitonin below 60ng/L was always associated to an intrathyroidal localization of MTC. Qi *et al.*[11] found at least 10 patients had slightly increased pre-Ct levels (such as 71.4 ng/L or lower) (normal <8.4 ng/L for male and <5.0 ng/L for female) they had intrathyroid MTC (including two C-cell hyperplasia) without lymph node metastases. Recently, Pelizzo *et al.* [22] points the biochemical (basal/stimulated) calcitonin testing was unable to ensure prophylactic thyroidectomy in 68% of cases, and it did not guarantee a definitive cure in any patients, particularly in the case of level ATA-B/C RET gene mutations. Thus, in this study, these cases still had consistently undetectable post-Ct (<2ng/L) and had no cervical abnormalities by imaging that may be considered clinical cured despite relatively late thyroid surgery (Due to ~5% of MTC cases with undetectable basal calcitonin levels who undergo TT may show abnormally high stimulated calcitonin values. These latter cases cannot be considered to be cured, and they should be followed up carefully. Therefore, a more accurate following stimulation
with combined calcium/pentagastrin infusion should be performed. However, some specialists argue that stimulated calcitonin testing is rarely needed in the diagnosis or follow-up of MEN2-related MTC, because new calcitonin assays have high sensitivity and specificity, and detection values are as low as 1-2 ng/L. (Tavares MR, Toledo SP, Montenegro FL, et al. Surgical approach to medullary thyroid carcinoma associated with multiple endocrine neoplasia type 2. *Clinics* (Sao Paulo), 2012;67:149-154.) (Kloos et al [5])

Q6: in several MTC cases, few neck lymph nodes were resected and analyzed (table 3)

A6: There were 6 cases received operation and 4 cases received lymph node dissection in table 3.

i) F5-Ⅲ4 (pre-Ct 24.3ng/L; tumor size 0.3cm/0.2cm) didn’t perform neck lymph nodes resection based on predictive testing for RET proto-oncogene mutation and basal serum calcitonin and our experience (Elisei et al 2012 [8]; Machens et al 2009 [10] and Qi XP et al 2013 [11]).

ii) F6-Ⅲ2 (LN-/resected, 0/3) and F6-Ⅲ5 (LN-/resected, 0/1) underwent TT associated with level VI central neck compartment dissection and had few neck lymph nodes resection, it speculated that the some Chinese people have relatively few number of cervical level VI lymph nodes (Lau GS, Lang BH, Lo CY, et al. Prophylactic thyroidectomy in Chinese patients with multiple endocrine neoplasia type 2A syndrome after the introduction of genetic testing. *Hong Kong Med J*, 2009; 15:326-331) or may missing some of lymph nodes in pathological examination.
Q7: if age was evaluated using the mean, then SD may be added (table 3); the same for tumor size (table 2).

A7: According to your suggestion, we have added SD in table 2 and in the text. (see page7, line14-15; page8, line17 and line19; page9, line17; page10, line8, 13, and 17; respectively)

Q8: Cases with 78y, F7-II 2/F and F3-III 9/F had neck LN metastasis ? and two cases calcitonin>60pg/ml were fully studied for the presence of metastasis?

A8: Thanks for your thoughtful comment. We have added the relevant content in the text. For F8-II 2/F (78y; tumor size 3.5cm; Ct 1758.0ng/L and CEA106.0 ng/ml), F7-II 2/F (tumor size 2.5cm; Ct 487.6ng/L; and CEA 25.9 ng/ml ) and F3-III9/F (tumor size 1.0cm; Ct 206ng/L; CEA12.2 ng/ml), the ultrasound scanning detected multiple lymph nodules in neck (1.8 cm, 1.1cm and 0.8cm, respectively). In Machens’ article (Machens A, Schneyer U, Holzhausen HJ, Dralle H. Prospects of remission in medullary thyroid carcinoma according to basal calcitonin level. J Clin Endocrinol Metab. 2005;90:2029-2034.), states “Nodal metastasis started emerging at pre-Ct levels of 10ng/L (reoperative setting) and 40 ng/L (primary setting) and at primary tumor diameters as small as 5 mm (primary and reoperative setting). Distant metastasis and extrathyroidal growth began appearing at pre-Ct levels of 150ng/L (reoperative setting) and 400ng/L (primary setting) and at primary tumor diameters of 12-15 mm (primary and reoperative setting)”. Lymph node metastasis in the neck and upper mediastinum is a frequent finding in MTC cases that are diagnosed late, and metastases are present in about 70% of MTC patients who have a palpable thyroid nodule (>1.0 cm diameter). (Tavares MR, Toledo SP, Montenegro FL, et al. Surgical approach to medullary thyroid carcinoma associated with multiple endocrine
neoplasia type 2. *Clinics* (Sao Paulo), 2012;67:149-154.) However, our previous data showed that at least 4 patients had increased pre-Ct levels (such as 162ng/L, 169ng/L, 519ng/L and 210 ng/L, respectively) by fully-automated chemiluminescence immunoassay they had intrathyroid MTC without lymph node metastases.(Qi et al.[11]) whereas 1 patients with 219.89 ng/L had fibro-adipose tissue invasion (metastasis). The reason of this appearance maybe the method of calcitonin detection (especially sensitive and accurate) should be considering, or the differences in MTC progression exist among individuals.In our study, we speculated F8- II 2/F and F7-II 2/F may exist neck lymph node metastasis, F3-III9/F should be highly suspected the presence of lymph node metastasis (they rejected to operation and perform cytological histopathological examination). The other two cases (F4 III2/F and F6-III5/F) had consistently undetectable Ct and no abnormal imaging post-operatively, that were considered clinical cured and also had no metastasis.

Q9: comment that MEN2B patient was diagnosed late despite typical clinical signs.

A9: Thanks for your comment. We have added the relevant content in the discussion. The basic causes of missed diagnosis and misdiagnosis is for a developing MEN 2 program in China, lack of awareness of this disease. The other reason is lack of attention the clinical relevance of non-endocrine manifestations (such as tearless crying, corneal fibers, neuromas of tongue and conjunctiva, ganglioneuroma of the submucous and myenteric plexus (IGNM), and various musculoskeletal stigmata giving rise to a “Marfanoid” habitus with hyperflexible joints, etc.) for early diagnosis of MEN 2B. Moreover, detection of RET gene and pre-Ct didn’t timely perform is also an important influencing factor.
Q10: page 4, line 18 – systemic or systematic?

A10: Thank you very much. It should use the “systematic” (see page 4, line 18).

Q11: parents and grand-fathers were personally investigated?

A11: Yes, all proband were diagnosed and treated at the Zhejiang Cancer Hospital or the 117th PLA Hospital. When proband were diagnosed MEN2, our team personally carried out the family screening (including their parents, grand-fathers and offspring).

Q12: Reference 8 (page 17) – please cut: Task F; begin with Kloos.

A12: Thank you. MS Reference “8”-5 have cut “Task F” (see page 16, line 14).