CHAPTER V

DISCUSSION AND CONCLUSION

We described six unrelated Thai individuals clinically suspected of RTH with one patient having a *de novo* p.M313T mutation in the *TRβ* gene. Even though the identified mutation has been previously reported in other populations, its functional consequence has not been investigated. Here we revealed that the mutation reduced T_3-dependent transcriptional activity and exhibited a dominant negative effect confirming its pathogenicity.

The finding of increased thyroxine (T_4) and tri-iodothyronine (T_3) in patients with normal or elevated thyroid stimulating hormone (TSH) can be a diagnostic dilemma. The differential diagnosis includes RTH, TSH-secreting pituitary adenoma, artefactual increase in levels of TSH due to heterophilic antibodies or thyroxine-binding protein abnormalities. Careful clinical and biochemical evaluation is therefore needed. However, after the responsible gene has been identified, genetic testing of RTH becomes possible and practical. Mutation analysis can provide an earlier definitive diagnosis of RTH, avoiding protracted and expensive pituitary function tests. It also enables mutation screening in at risk family members.

In addition, previous studies revealed families with the phenotype of RTH in the absence of mutations in the *TRβ* gene. This was called non-TR RTH. Given the complexity of thyroid action, there are several possibilities that may be linked to non-RTH. Dumitrescu *et al.* (2006) described that these could be due to defects in 1) thyroid hormone entry into cells (B), 2) its intracellular metabolism and distribution (C), 3) cytosolic (non genomic) effects (E), 4) translocation into the nucleus (D), 5) association with the receptor (A) and 6) abnormalities in co-regulators or other post receptor effects required for the proper mediation of thyroid hormone action (F) (figure 24) [51]

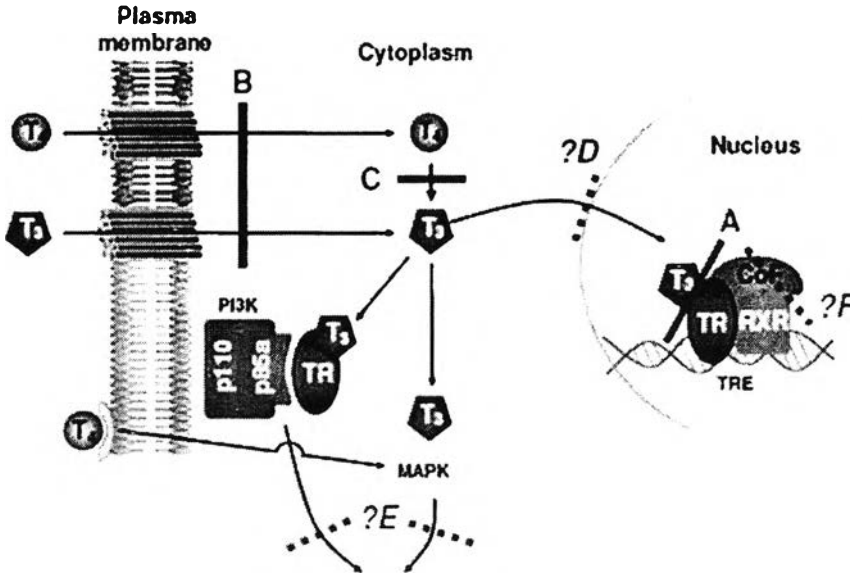

Figure 24 Schematic drawing represents defects in thyroid hormone action. Shown in red are reported (straight lines A, B, C) and putative (dotted line, D, E, F) defects.
[http://www.hotthyroidology.com/_css/thyruidology.css" type=text/css rel=stylesheet][51]

Recently, *MCT8* (X-linked monocarboxylate transporter 8) [52-55] and *SBP2* (SECIS-binding protein) mutations were reported. The *MCT8* gene encodes for thyroid hormone transporter and *SBP2* gene encodes for a component of the machinery leading to the synthesis of selenoproteins involving in thyroid hormone metabolism. Therefore, defects of these genes responsible for the transportation of thyroid hormone into cells resulted in deiodinase deficiency in convertion of T_4 to T_3. Nevertheless, these two deficiencies had different patterns of inheritance and thyroid hormone concentrations in serum from RTH as shown in table 15. The MCT8 defect has an X-linked inheritance, and the SBP2 defect has an autosomal recessive inheritance[51]. These suggested that they did not associate with non-TR RTH. Studies of cofactors that are involved in T_3-action have not detected causative mutations in RTH patients[6,57]. However, further investigation of defects in translocation into the nucleus (D) and cytosolic (non genomic) effects (E) is necessary to further elucidate the underlying mechanism of non-TR-RTH.

Table 15 Summary of thyroid function test abnormalities in syndromes of reduced sensitivity to TH[51].

	TRβ defect	**MTC8 defect**	**SBP2 defect**
FT_3	NI or ↑	↑	NI or↓
FT_4	↑	NI or↓	↑
TSH	NI or ↑	NI or ↑	NI or ↑

The p.M313T mutation identified in a Thai patient with RTH has been previously reported in other families with RTH but never been investigated for functional significance. These patients had different clinical findings varied from euthyroid to thyrotoxicosis.The variation of RTH phenotype among different families harboring the same mutation suggests that other factors including genetic background may be important in the expression of the phenotype[8,16]. This study also revealed that the p.M313T mutation had a significantly decreased T_3 transactivation activity and had a strong dominant negative effect at 10^{-7}M of T_3. Therefore, we provide evidence supporting that the p.M313T is a disease-causing mutation.

The p.R316H mutation which is located close to the p.M313T was investigated for its functional consequence. The transient cotransfection study using the positively-regulated Palx3 Luc reporter showed that the R316H had a weak dominant negative effect which was consistent with the previous report by Hayashi *et al.* [56]. Conversely a study by Geffner *et al.* reported that the R316H did not have a domimant negative effect when using a positively-regulated MTV-TRE_{ir}-CAT reporter even though it caused a significant impairment in T_3-binding activity [49]. The different results from the latter study could be from a different technique or reporter assay.

Five uncharacterized mutations in the *TRβ* gene (I276L, I280S, L330S, G344A, M442T) [43-47] identified in patients with RTH were also analyzed for their functional significance. All mutations except the I276L and G344A caused a significant impairment of T_3-dependent transactivation activity. Nevertheless, all mutants exhibited dominant negative effect in the presence of 10^{-7}M of T_3.

The reason that the I276L and G344A mutants retained their T_3-dependent transactivation activity could be a reversible conformation making them able to activate expression of the target gene in the presence of 10^{-7}M of T_3. A previous study by C.-W. Lam *et al.* showed that the regression equation of T_3 binding affinity by the I276L

mutant was 0.7 fold of that of the wild-type *TRβ*[45]. Consistent with its mild effect on the protein function, the patient with the I276L had mild RTH [45].

Another mutation with normal T_3 dependent transcriptional activity but with dominant negative effect was the p.G344A mutation. Kvistad *et al.* reported a patient with the G344A mutation who had mild RTH with increased levels of FT_3 and FT_4 and normal TSH [46]. We showed that the pathogenicity of this mutation could be its dominant negative effect, not the impairment of T_3 dependent transactivation activity.

Our studies confirmed the dominant negative effect as a major mechanism for RTH. Some causative *TRβ* mutations can preserve their T_3 dependent transactivation activity with a dominant negative effect.

In conclusion, this study has identified a Thai patient with a *de novo* p.M313T mutation in the *TRβ* gene and further elucidated its pathogenic mechanism. In addition, five different uncharacterized known mutations were explored. Even though not all of the mutant TRβ1s exhibited a significant impairment of T_3-induced transactivation, all these mutant TRβ1s showed a dominant negative effect on cotransfection with the wild-type TRβ1. Our studies provide a strong support that interfering with the T_3-mediated transcriptional activation of the wild-type TRβ1 independent of the inability to activate transcription is a major pathogenic mechanism causing RTH.