CHAPTER II

BACKGROUND AND LITERATURE REVIEWS

1. Resistance to thyroid hormone (RTH)

Resistance to thyroid hormone (RTH) is an inherited syndrome of tissue hyporesponsiveness to thyroid hormone (T_3). It is caused by mutations in the *thyroid hormone receptor (TR)-β* (*TRβ*) gene[4]. Patients with RTH have elevation of circulating free T_4 (FT_4) and free T_3 (FT_3) levels with persistence of normal or slightly elevated serum TSH levels. The clinical phenotype can vary both between different families and between affected family members[6], which may be due to the severity of the hormonal resistance, effectiveness of compensatory mechanisms, modulating genetic factors, and effects of prior therapy[1, 2]. Some patients can have mixed clinical features of hypo- and hyperthyroidism, suggesting variable resistance in different tissues[6]. Variable resistance in different organs due to differences in the distribution of TRα and TRβ can cause a mosaic of hyper- and hypothyroid signs in the same patient[1, 2, 9]. The most common findings include goiter, growth retardation, attention deficit and hyperactivity disorder (ADHD), and hyperthyroid cardiac symptoms [1-3, 10].

2. Clinical classification

Before mutations in the *TRβ* gene causing RTH were identified, the diagnosis of RTH was entertained on the basis of elevated levels of serum thyroid hormones and nonsuppressed TSH. RTH can be divided clinically into two entities: generalized resistance to thyroid hormone (GRTH) and central or pituitary resistance to thyroid hormone (PRTH)[3]. Currently, molecular techniques have increased our understanding of RTH. The *TRβ* gene generates two TRβ isoforms by alternative exon utilization results in TRβ1 and TRβ2 isoform[11]. The TRβ1 isoform is ubiquitous in peripheral tissues[12] and the TRβ2 isoform is found predominantly in the anterior pituitary and brain[13, 14]. In 1997 Safer described the defective in TRβ1 and TRβ2 isoform are mediating for GRTH and PRTH syndrome respectively[12].

Thus, the diagnosis of RTH can be divided into two groups by based on the clinical findings, standard laboratory tests and confirmed by genetic studies.

1. Generalized resistance to thyroid hormone (GRTH): Besides thyroid hormone resistance at the level of the pituitary and hypothalamus, there is also peripheral tissue resistance to thyroid hormone. Elevated thyroid hormone concentrations with near normal TSH can result in signs of hypothyroidism or euthyroidism in patients [3, 12].

2. Central or pituitary resistance to thyroid hormone (PRTH): There is thyroid hormone resistance at the level of the pituitary and hypothalamus. However, peripheral tissues are sensitive to T_3. Patients with PRTH tend to exhibit features of hyperthyroidism such as agitation or sinus tachycardia. This is because while peripheral tissues retain their normal sensitivity to thyroid hormones, higher concentrations of thyroid hormones are needed to evoke a response at the level of the pituitary [3, 12].

3. Incidence and inheritance

The precise incidence of RTH is unknown. Because routine neonatal screening programs are based on the determination of TSH, RTH is rarely identified by this means[3]. A limited neonatal survey by measuring serum T_4 concentration has suggested the occurrence of one case per 40,000 to 50,000 live births [3, 7, 8]. Most reported cases of RTH are inherited in an autosomal dominant inheritance pattern, with the exception in only one patient showing an autosomal recessive inheritance pattern in one family [1, 5, 6].

4. Etiology and molecular genetics of RTH

4.1 Thyroid hormone receptor structure

TRs are encoded by the *TRA* and *TRβ* genes located on chromosomes 17 and 3 respectively in humans[15]. Alternative splicing from the *TRA* gene generates TRα1 and TRα2 isoforms[16], whereas the *TRβ* gene generates TRβ1 and TRβ2 isoforms by separate promoter usage and alternative splicing in the 5′ region resulting in distinct TRβ1 and TRβ2 protein N-termini (figure 1)[11].

A previous study reported the structure of the human *TRβ* gene. From the figure 1, the *TRβ* gene consists of 10 exons. Exons 1, 2 and the first 44-bp of exon 3 make up the 5′-UTR; the remainder of exon 3 and exons 4–10 encode TRβ1 protein (figure 1A)[11]. Thus, the cDNA sequence is 1.386 kilobases long and appears to consist of 8 exons (figure 1A).

For *TRβ2*, the exon 1 of *TRβ* is a region of 5′-UTR and a specific exon 1 of TRβ2, exons 5-10 are the coding region for TRβ2 protein (figure 1A). The cDNA sequence is 1.1 kilobases long and appears to consist of 6 exons[11].

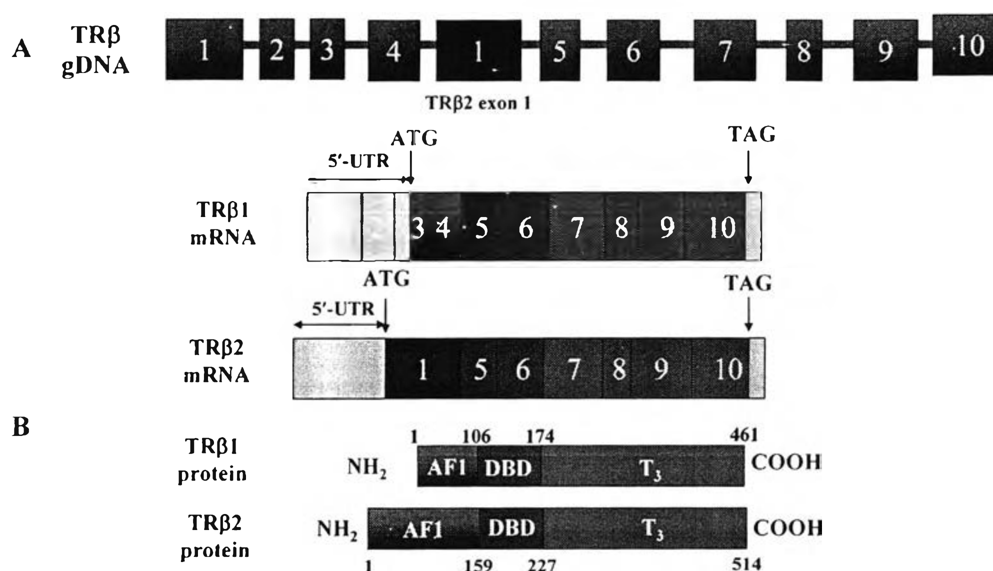

Figure 1 Structure of the human *TRβ* gene[11].
Panel A: Exons 3-10 encodes for TRβ1. A specific exon 1of TRβ2 between exon 4 and 5 of *TRβ* gene and exons 5-10 encodes for TRβ2.
Panel B: The distinct of N-termini resulting in the specific of TRβ1 and TRβ2 proteins.
Note that: TRβ1 and TRβ2 are transcribed from the same gene, with separate promoter usage. Alternative splicing generates TRβ1 and TRβ2 isoforms.

The expression of TRs is tissue-dependent and developmentally regulated[15, 16]. Almost all tissues express the TRα1 and TRβ1 isoforms, but TRα1 isoform is expressed predominantly in the heart, bone and brain[14, 17, 18], whereas TRβ1 isoform is more abundant in the liver, kidney and thyroid gland[14, 17]. The expression of TRβ2 isoform is limited to the pituitary, hypothalamus, retina and inner ear[14].

4.1.1 Thyroid hormone receptor and functional domains

TRs are crucial mediators for thyroid hormone action, which regulate T_3-target gene transcription and are members of a large family of nuclear receptors that include the steroid hormones. They function as ligand-dependent transcription factors. Like other nuclear receptors, TRs have modular structures with the main functional domains including (figure 2) [6, 19]:

1. Transactivation domain at the amino terminus is a region for interaction with basal transcriptional factors to form complexes that repress or activate transcription. This domain is highly divergent between TRα and TRß isoforms suggesting differential roles in transcriptional regulation.

2. DNA binding domain (DBD) in the middle of the molecule is a region to bind specific sequences or promoter sequences in target genes (known as hormone response elements) which is crucial for TR function. This domain is the most conserved region of the nuclear receptor superfamily.

3. Hinge region (H) between the DBD and LBD contains an amino acid sequence that is associated with nuclear localization which is important for T_3-mediated translocation of TR into the nucleus. It is also associated with binding of corepressor proteins[16].

4. Ligand-binding domain (LBD) at the C-terminus is a critical region for T_3 binding, dimerization and basal repression by unliganded TR.

The DNA binding domains of the different receptor isoforms are very similar. However, they are divergent among transactivation and ligand-binding domains. All receptor isoforms except the TRα2 can bind T_3. The TRα2 has a unique carboxy-terminal region that does not bind T_3 [1, 6] (figure 2B).

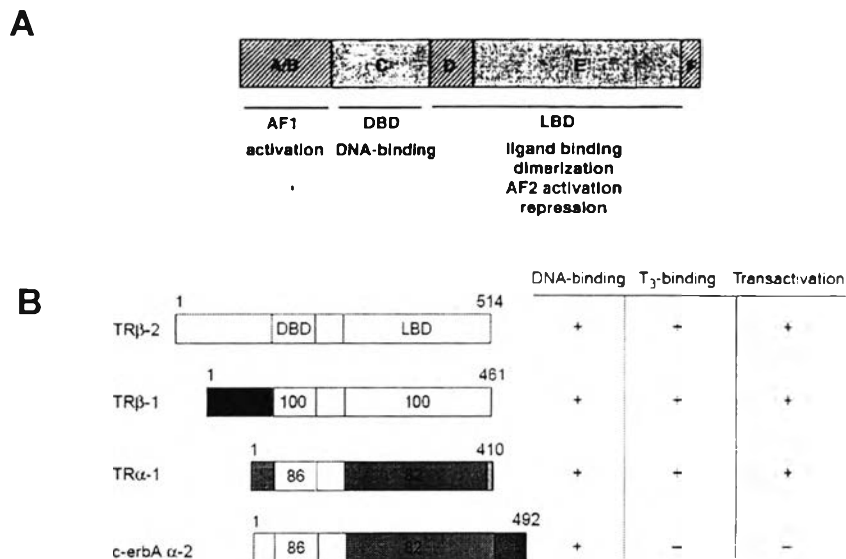

Figure 2 The thyroid hormone receptor (TR) structure.
Panel A shows the main functional domain of TR[19].
Panel B shows comparison of amino acid homologies and their functional properties. The lengths of the receptors are indicated just above receptor diagrams and the % amino acid homology with TRβ2 is included in the receptor diagrams[6].
Abbreviations: DBD, DNA-binding domain; LBD, ligand-binding domain; T_3, triiodothyronine.

4.2 Molecular mechanisms of T_3 action for gene expression regulation

The actions of T_3 are mediated by nuclear thyroid hormone receptors (TRs). When activated, TRs bind to the T_3-response elements (TREs) located in the promoters of the target genes.

4.2.1 Thyroid hormone response elements for TRs

The main function of the TRs as a transcription factor is to regulate target gene expression by recognizing and directly binding to specific response element sequences in the promoters of the T_3- target genes (T_3 response elements; TREs) causing transcriptional activation or repression (figure 3A)[1]. The TRE is composed of two consensus hexamer half site sequences of AGGTCA separated by spacing nucleotides[16]. The half sites can be arranged as palindromes (TREpal), direct repeats (DRs), and inverted palindromes (IPs) (figure 3B). The optimal spacing is zero for TREpal0, four for DR4, and six nucleotides for IP6[16, 19]. These different orientations of TREs are required for specific binding of TRs to TREs of the target genes[16].

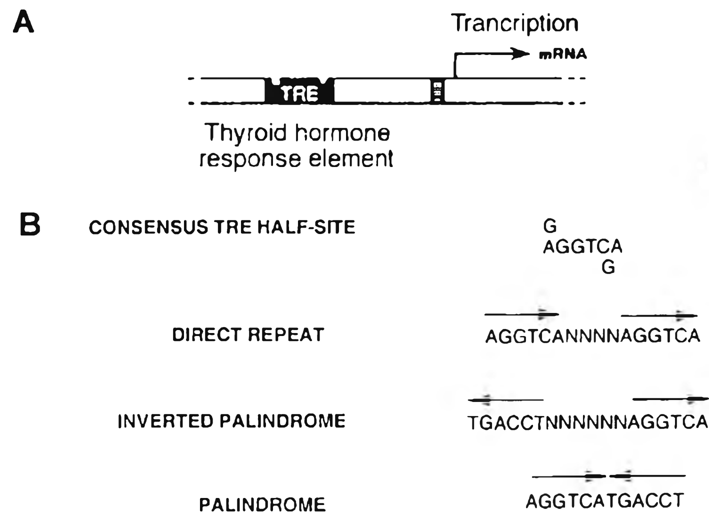

Figure 3 Schematic representation of the elements involved in the mediation of thyroid hormone action through the regulation of target (T_3-responsive) gene expression.
Panel A shows the position of TRE. The TRE is in the promoter region of the T_3- target gene[1].
Panel B shows half-site orientation and optimal nucleotide spacing between half-sites[16].
Note that: N refers to nucleotides, and arrows show direction of half-sites on the sense strand. TRE, thyroid hormone response element.

Additionally, the DNA-binding domain containing two zinc fingers each composed of four cysteines coordinated with a zinc ion (figure 4) [16]. Within the first zinc finger, there is a P box region in the carboxyl terminus mediating the half-site sequence recognition by directly contacting the major groove nucleotides of TRE[19]. It is important that the P box can distinguish a single base change in the half site used by two subfamilies of the receptors (AGGACA for the glucocorticoid receptor versus AGGTCA for the estrogen and thyroid receptors)[19, 20]. The second zinc finger has an A box region interacting with the minor groove nucleotides of TRE. Thus, the zinc fingers mediate specificity in binding to TREs [16].

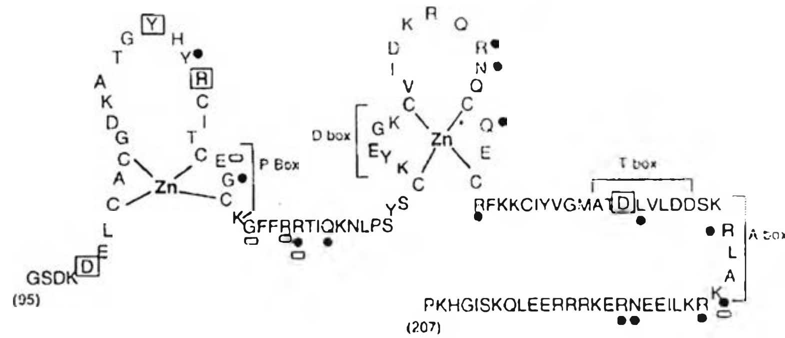

Figure 4 Schematic drawing of the two zinc fingers of human TRβ and the various subregions within the DNA-binding domains. Squares, TR/RXR heterodimerization contacts; ovals, direct base contacts; solid circles, direct phosphate contacts[16].

4.2.2 Thyroid hormone receptor complexes

TRs can bind to TREs as monomers, as homodimers or as heterodimers (figure 5). Previous studies reported that TRs heterodimerized with proteins from pituitary and liver nuclear extracts. These proteins were called TR auxiliary proteins (TRAPs) and enhanced TR binding to TREs. Because these proteins were expressed in nuclear extracts from many different tissues and species, TR/TRAP heterodimers could be formed in all cells that contain TRs. Because these heterodimers appeared to bind better to TREs than TR homodimers, it was speculated that they played a role in T_3-regulated transcription [16].

Several lines of evidence have suggested that retinoid X receptors (RXRs) are the major endogenous TRAPs and play a critical role in T_3-mediated transcription [21,22]. This TR/RXR heterodimer formation is a major form that increases efficiency and stabilizes binding affinity of TRs to TREs on DNA sequences of the target genes (figure 5) [16].

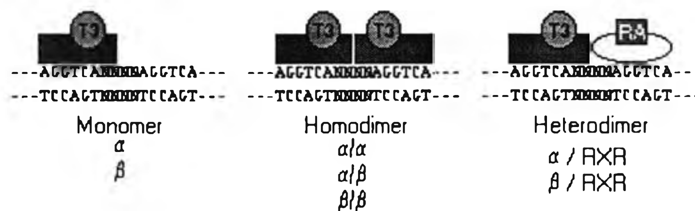

Figure 5 Schematic drawing represents the dimerization forms of TRs in TREs. [http://www.vivo.colostate.edu/hbooks/pathphys/endocrine/thyroid/receptors.html]

4.2.3 Transcriptional regulation by TRs.

TRs bind to hormone response elements both in the absence and presence of hormone binding. TRs can positively and negatively regulate target gene transcription (figure 6) [1, 6].

Positively regulated genes

In the absence of T_3, the unliganded TRs repress or "silence" transcription in a process that involves TR interaction with a corepressor complex. Binding of T_3 releases corepressors, relieving silencing and inducing the recruitment of coactivators that mediate transcriptional stimulation (figure 6A)[1].

Examples of proteins positively regulated by T_3

- Fatty acid synthetase, growth hormone, lysozyme silencer, malic enzyme, moloney leukemia virus enhancer, myelin basic protein, myosin heavy chain α, phosphoenolpyruvate carboxykinase, RC3, and spot 14 lipogenic enzyme and type I 5′-deiodinase[23-26].

Negatively regulated genes

In the absence of T_3, the unliganded TRs activate transcription in a process that involves corepressors. Addition of T_3 dissociates corepressors and recruits coactivators. In the case of negatively regulated genes, this T_3-mediated exchange of corepressors and coactivators inhibits transcription (figure 6B)[1].

Examples of proteins negatively-regulated by T_3

- Epidermal growth factor receptor, myosin heavy chain β, prolactin, thyroid-stimulating hormone α, thyroid-stimulating hormone β, thyrotropin-releasing hormone, and type II 5′-deiodinase

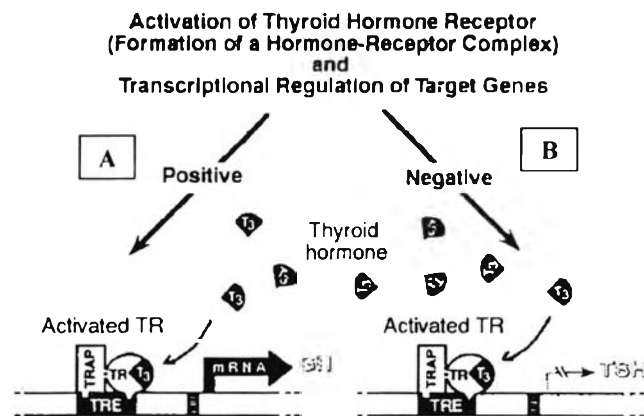

Figure 6 TH receptor-mediated transcriptional silencing and activation[1].
Panel A shows positively regulated genes.
Panel B shows negatively regulated genes.

4.2.3.1 The molecular complexity of T_3 action on positively regulated genes

T_3 binding is associated with a conformational change in the receptor that causes it to function as a transcriptional activator and as a transcriptional repression in the absence the hormone ligand binding. Thus, the biological effects of TRs to activate expression of target genes depend on T_3 binding state (figure 7). This is the case for positively regulated genes known as T_3-dependent transactivation function as described below.

1. T_3-free state: TRs heterodimerize with RXR at the transactivation domain and bind TRE, as a conformation that promotes interaction with a group of transcriptional corepressor molecules. There are two major corepressors involved in transcriptional repression including nuclear receptor corepressor (NCoR) and silencing mediator for retinoid and thyroid hormone receptors (SMRT)[6,16, 19,27,28].

2. Ligand-bound state: Binding of T_3 to TR/RXR induces a conformational change and releases the corepressor complex making it competent to bind a group of coactivator proteins. There are at least two major complexes involved in ligand-dependent transcriptional activation including the steroid receptor co-activator (SRC) complex and the vitamin D receptor interacting protein–TR associated protein (DRIP–TRAP) complex[6,29,30]. SRCs also interact with the CREB-binding protein (CBP), the coactivator for cAMP-stimulated transcription, in addition to the related protein p300,

which interacts with the viral co-activator E1A[6,29]. Additionally, CBP/p300 can interact with P/CAF (p300/CBP-associated factor), the mammalian homolog of a yeast transcriptional activator[6,30,31]. P/CAF has intrinsic histone acetyltransferase (HAT) activity. P/CAF and CBP interact with TBP associated factors (TAFs) and RNA pol II, respectively. Thus, P/CAF and CBP have dual roles as adaptors of nuclear receptors to the basal transcriptional machinery and as enzymes (HAT activity) that can alter chromatin to euchromatin to facilitate the assembly of basal transcriptional machinery and increase the transcription rate or turn on the target genes. DRIP–TRAP components are mammalian homologs of the yeast mediator complex, which associates with RNA Pol II[6,32,33]. Thus, TR recruits the DRIP–TRAP complex which, in turn, can recruit or stabilize the RNA Pol II holoenzyme via their shared subunits. Recent chromatin immunoprecipitation assays of proteins bound to hormone-response elements (HREs) have suggested that there might be a sequential, perhaps cyclical, recruitment of coactivator complexes to HREs[6, 19,34,35].

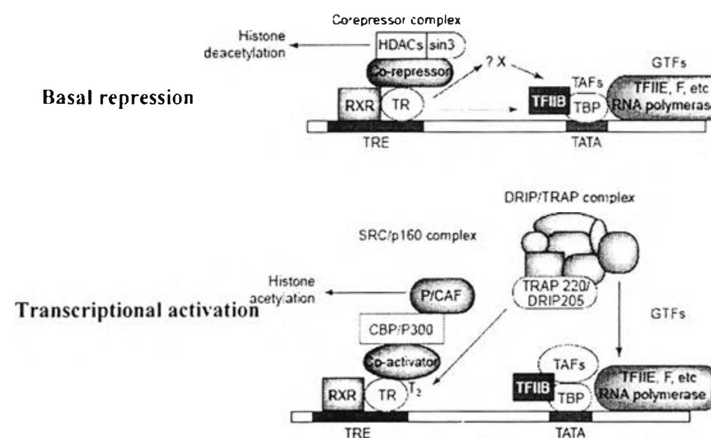

Figure 7 Model for molecular mechanisms of thyroid hormone receptor action[6].
CBP, cAMP-response element-binding protein; DRIP, vitamin D receptor- interacting protein; GTF, general transcription factor; HDAC, histone deacetylase; P/CAF, p300/CBP-associated factor; RXR, retinoid X receptor; SRC, steroid receptor coactivator; TAF, TATA-binding protein-associated factor; TBP, TATA-binding protein; TF, transcription factor; TR, thyroid hormone receptor; TRAP, TR-associated protein; TRE, thyroid hormone-response element (31).

4.3 Molecular pathogenesis of RTH

Dominant negative effect (DNE) is a hallmark of RTH pathogenesis that the mutant TRs inhibit the wild-type TRs function. Most mutations found in the ligand-binding domain (LBD) were clustered on exons 7-10[6] (hot spot region) of *TRβ*. These mutations are nucleotide changes that result in single amino acid substitutions. Nucleotide deletions or insertions and nonsense mutations, which cause truncation as a result of premature termination of translation, have also been identified[36] (figure 8).

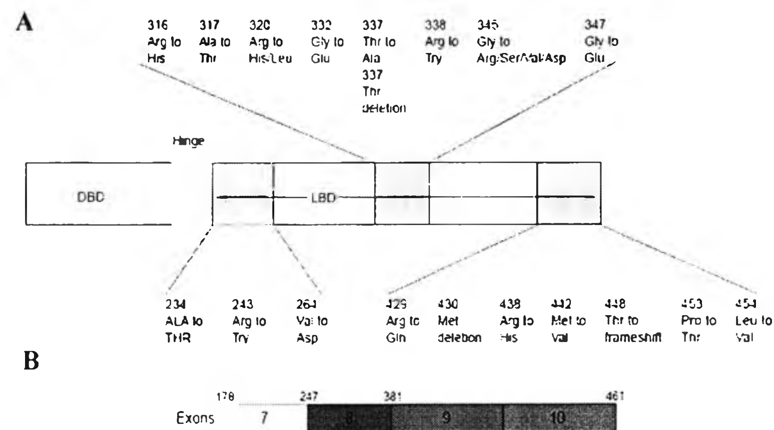

Figure 8 The hot spot regions in the ligand-binding domain of TRβ[6].
Panel A shows *TRβ* mutations clustering on three major ligand-binding domains.
Panel B shows TRβ1 exon organization in ligand-binding domain.

Analysis of the *TRβ* gene from the published case reports showed that the affected members had deletions of the entire coding region of both TRβ alleles but normal *THRA* genes[6,37]. Some families have heterozygous members who had only one deleted TRβ allele exhibited a normal phenotype, with normal thyroid function tests. This observation suggests that a single copy of TRβ is sufficient for normal function and debates against haplotype insufficiency[6]. Instead, mutant TRβ derived from the abnormal allele probably has dominant-negative activity on the function(s) of normal TRs[1,6,38]. There is only one patient with severe RTH and mental retardation was the consanguineous product of parents. This patient was found to be homozygous for mutations in both TRβ alleles, whereas his parents were found to be heterozygous[6] (figure 9).

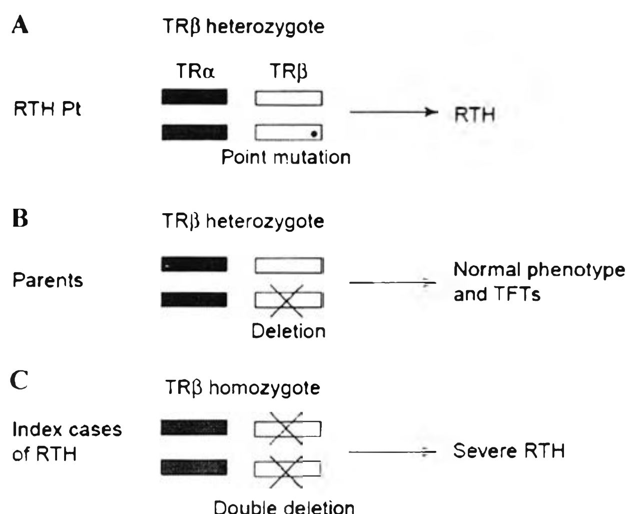

Figure 9 Clinical and genetic observations in the RTH families provide evidence for the pathogenic mechanism of RTH[6].
Panel A shows a point mutation in the *TRβ* gene causing RTH.
Panel B shows a deletion of one *TRβ* allele giving normal phenotype.
Panel C shows deletion of both TRβ alleles in patients gives severe RTH phenotype.

4.3.1 Mechanisms for dominant negative activity

The molecular mechanism of the DNE is not fully understood. From the previous reports, the possible mechanisms of DNE are:

1. Formation of inactive dimers

Mutant TRβs form dimers with wild-typeTRβs resulting in tritration out of the remaining functional TRβs and transcriptionally inactive dimers to repress T_3-mediated transcription (figure 10A)[38].

2. Competition between wild-type and mutant TRβs at the T_3 response element (TRE) by transcriptionally inactive complexes

Mutant TRβs that are transcriptionally inactive can competitively block normal TRβs by forming homodimers and bind to TREs. Moreover, mutant TRβs can form heterodimers with RXRs on TREs. Thus, these inactive complexes block T_3-mediated transcription (figure 10B)[38].

3. Titration out of common nuclear factor

Mutant TRβs sequester some transcriptionally important factors which are limiting in the cells. This possibility is considered to be highly likely because, recently, many transcriptional co-activators have been identified (figure 10C)[38].

4. Impaired corepressor release

Mutant TRβs are defective in ligand-induced release of corepressors which have been shown to have strong dominant-negative activity. The gel shift assay showed that mutations in the hinge region of TRβs failed to dissociate from the corepressors except at very high T_3 concentrations (figure 10D)[39].

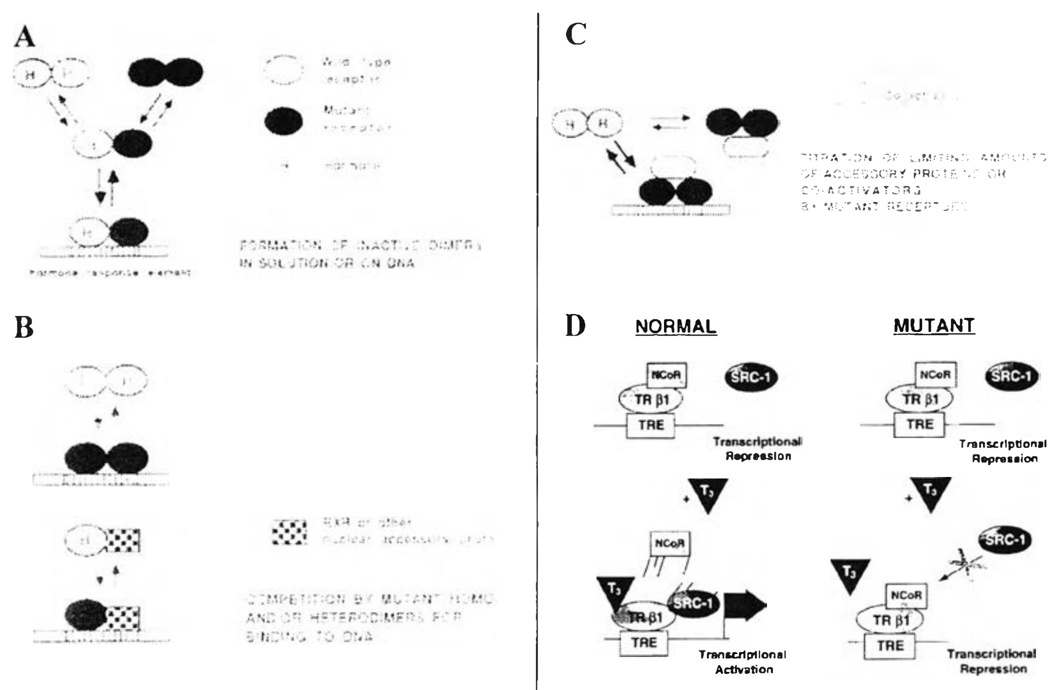

Figure 10 Models for mechanisms of dominant negative activity by mutant TRs.

4.3 RTH in patients without *TRβ* mutations (Non-TR RTH)

Some patients with RTH can occur in the absence of mutations in the *TRβ* gene[40]. The clinical manifestations and laboratory abnormalities in these patients are not different from those with mutations in the *TRβ* gene[17]. The molecular basis of non-TR RTH is still unknown. A study by linkage analysis revealed that TRα did not associate with non-TR RTH[6]. In addition, mutations of cofactors involved in T_3-action have not been reported in RTH patients[41].

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5. Role of diagnostic procedures in RTH

The clinical presentation of RTH is highly variable and there are some disorders with elevated FT_3 and FT_4 levels together with non-suppressed TSH as shown in table 2 .

Table 2 Differential diagnosis of conditions associated with an elevated FT_3 and FT_3 levels[3].

	FT_3	**FT_4**
Resistance to thyroid hormone	↑	↑
TSH-secreting pituitary tumour	↑	↑
Interfering antibodies to thyroid hormones	↓ or N	↑
Thyroxine replacement therapy	↑	↑ or N
Familial dysalbuminaemic hyperthyroxinaemia	↓ or N	↑

*N=normal

Thus, the precise diagnosis of RTH can be confirmed by genetic analysis. Identification of mutations in the *TRβ* gene will lead to correct diagnosis and appropriate family counseling including therapy and ultimate prevention.