REVIEW ARTICLE

Genetic features of thyroid hormone receptors

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Abstract

Thyroid hormone receptors (TR) are prototypes of nuclear transcription factors that regulate the expression of target genes. These receptors play an important role in many physiological processes. Moreover, a dysfunction of these proteins is often implicated in several human diseases and malignancies. Here we report genetic variations and alterations of the TRs that have been described in the literature as well as their potential role in the development of some human diseases including cancers. The functional effects of some mutations and polymorphisms in TRs on disease susceptibility, especially on cancer risk, are now established. Therefore, further investigations are needed in order to use these receptors as therapeutic targets or as biological markers to decide on appropriate forms of treatment.

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Introduction

Nuclear receptors are ligand-dependent transcription factors that regulate the expression of target genes. They play a crucial role in homeostasis, reproduction, development and metabolism by controlling the proliferation, differentiation, survival and apoptosis (Werner *et al.* 2004). Nuclear receptors, especially thyroid hormone receptors (TRs), are also involved in pathogenesis and in the development of several types of human diseases and malignancies.

It is known that mutations of TRs genes are the main cause of resistance to thyroid hormone syndrome (Rosen and Privalsky 2009). Further, genetic alterations and/or aberrant expression of the TRs are reported to be associated with human malignancies such as breast, liver, thyroid, pituitary and renal cancers (Wallin *et al.* 1992; Bronnegard *et al.* 1994; Puzianowska-Kuznicka *et al.* 2002; Silva *et al.* 2002; Crescenzi *et al.* 2003; Takano *et al.* 2003). Moreover, it was shown that there are certain numbers of functional polymorphisms of TRs that are strongly associated with diseases, including cancer (Rebai *et al.* 2009), autoimmune disease (Tassi *et al.* 1995) and coronary heart disease (Goumidi *et al.* 2011b), and with response to drugs (Duan *et al.* 2012). The contribution of genetic polymorphisms or alterations in the thyroid hormone receptor genes in diseases, including cancer, has been the subject of increasing interest. Therefore, in this report, we have reviewed the genetic status of TRs genes as well as their potential role in the development of some human diseases especially cancer.

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Thyroid hormone receptor genes

The TRs control essential functions in growth, development and metabolism, and are important for normal functioning of almost all tissues (Werner *et al.* 2004). TRs are encoded by two genes, *THRA* and *THRB*, located on chromosomes 17 and 3, respectively (Cheng 2000).

In human, these two homologous genes produce four types of receptors. An alternative splicing at the exon 9 of the *THRA* gene generates two mRNA encoding proteins, TRα1 (410 aa.) and TRα2 (492 aa.), which differ in their C-terminal region. Indeed, the isoform α1 is generated by an mRNA that extends from exon 1 to the end of the 3′ region of exon 9 (the stop codon is located in exon 9), while the isoform α2 is generated by alternative splicing that uses a donor site located at 128 bp after the start of the exon 9 and an acceptor site in exon 10 (the stop codon of this isoform is located at 364 downstream of the exon 10). According to this phenomenon, the isoform TRα2 has the distinction of not being able to fix the 3,5,3′-triiodothyronine (T_3), since the sequence of 40 amino acids of TRα1 (necessary to link to this hormone) has been replaced by another sequence of 120 amino acids specific to the isoform α2 (Laudet *et al.* 1991).

For *THRB* gene, the use of two alternative promoters generates two isoforms, TRβ1 (461 aa.) and TRβ2 (514 aa.), which differ in their N-terminal region (Williams and Brent 1995).

In fact, the mRNA of the isoform β1 is transcribed from exons 1 to 8, while the isoform β2 is transcribed from exons 5 to 10 using an alternative promoter in intron 4 (Frankton *et al.* 2004).

Each TR isoform has a specific tissue expression that varies with the stage of development (Cheng 2000; Yen 2001). In fact, although TRα1 and TRβ1 have ubiquitous expression, TRα1 is primarily expressed in heart, bone and brain, while TRβ1 is more abundant in liver, kidney and thyroid (O'Shea *et al.* 2003; Wondisford 2003). The expression of TRβ2 is limited to the pituitary, hypothalamus, retina and inner ear (Wondisford 2003).

Thyroid hormone receptor polymorphisms and association studies

THRA gene polymorphisms

Various polymorphisms in *THRA* gene have been identified and some of them have been studied regarding their relationship with specific diseases such as cancer, neurological diseases and cardiovascular diseases, and with normal variations. The markers investigated in these studies are presented in figure 1a.

A recent study, searching for the relationship between the SNP rs939348 and coronary heart disease, showed a significant association of this polymorphism and systolic blood pressure and the risk of hypertension, but not with coronary heart disease (Goumidi *et al.* 2011b). Individuals carrying the T allele of this SNP had higher systolic blood pressure than CC individuals. A previous study, investigating the role of circadian gene polymorphisms in behaviour diseases, also found a positive correlation between this polymorphic site and bipolar disorder (Kripke *et al.* 2009). However, this variant was not associated with variation of serum thyroid, stimulating hormone (TSH) level in healthy subjects (Lopez *et al.* 2008) and with Alzheimer's disease (Goumidi *et al.* 2011a), although subjects bearing, the TT genotype of this SNP had a tendency to have a higher risk of developing Alzheimer's disease. Further, Sørensen *et al.* (2008), related two polymorphic sites of *THRA* gene (rs12939700 and 2390 A/G) with TSH level, but no significant association has been found. The SNP rs939348 and two other polymorphic sites (rs1568400 and rs3744805) of the *THRA* gene were included in meta-analyses searching for the role of genetic variants of the genes involved in the complex regulatory mechanism determining the bio-availability of T_3 in osteoarthritis. No significant association was found (Meulenbelt *et al.* 2011).

Additional studies concern the polymorphic dinucleotide CA-repeat in the noncoding part of exon 9, which was tested for an association with thyroid cancer risk. Onda *et al.* (2002) have shown that a less aggressive thyroid cancer was found to be linked to increased thyroid hormone receptor expression and an expanded *THRA* microsatellite. Thus, they have suggested that the size of the microsatellite may influence

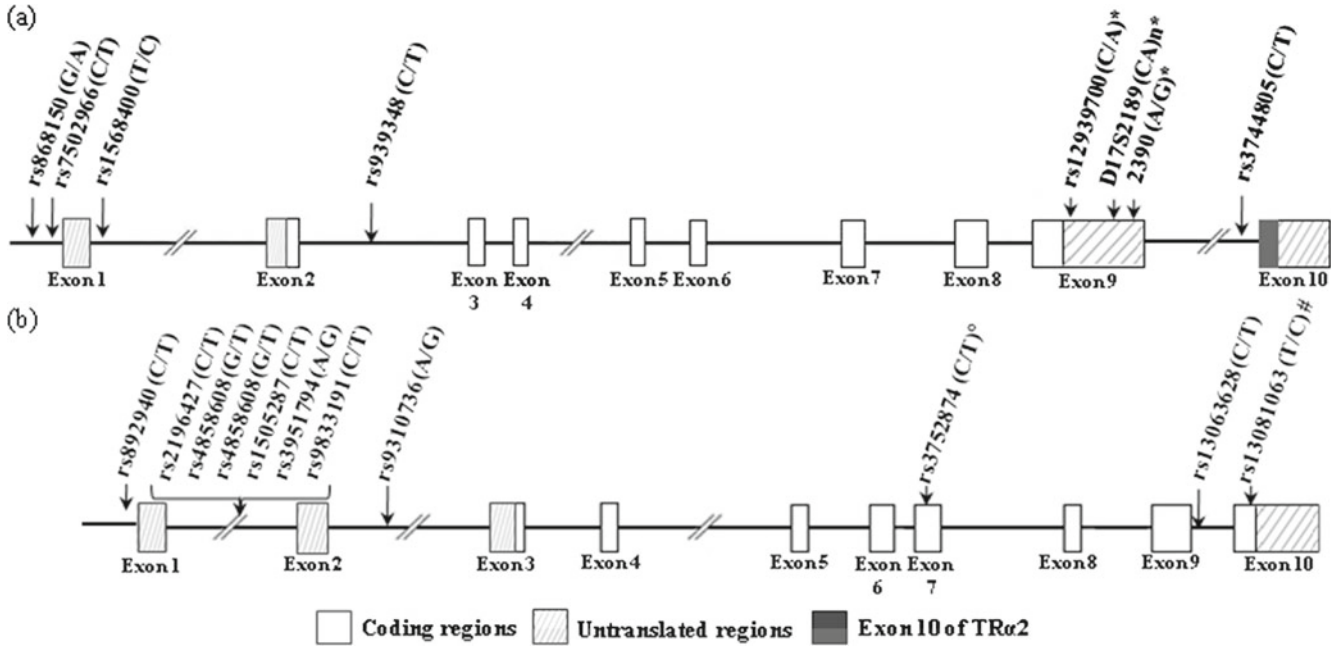

Figure 1. Schematic representation of (a) *THRA* and (b) *THRB* gene polymorphisms investigated in association studies. *, located in noncoding part of exon 9 of TRα1 isoform, °, rs3752874 is a synonymous SNP (F245F), #, rs13081063 is a synonymous SNP (F417F), SNPs studied by Sørensen *et al.* (2008): rs12939700 and 2390 (A/G) in *THRA* gene, rs13063628 and rs3752874 in *THRB* gene, SNPs studied by Goumidi *et al.* (2011a): rs939348, rs868150, rs7502966, rs1568400 and rs3744805. Only SNPs that were positively associated in genomewide analyses are presented in this figure.

the splicing phenomenon and receptor isoform expression since this marker is located near a splice junction (Onda *et al.* 2002). Later, Baida *et al.* (2005) a nd Rebai *et al.* (2009) failed to find an association between this polymorphism and thyroid cancer risk, but they suggested that the short alleles of the microsatellite may have a protective effect in thyroid cancer risk.

Also, *THRA* gene polymorphisms have been included in recent genomewide association studies with different diseases (Reiman *et al.* 2007; Lopez *et al.* 2008; Li *et al.* 2008; Giedraitis *et al.* 2009; Duan *et al.* 2012). Among these studies, only that of Duan *et al.* (2012) found a significant association between a single nucleotide polymorphism (SNP) and bronchodilator response (BDR) in asthmatics. In fact, in this study, the authors tested the association of 1116 SNPs across transcription factor genes with BDR in asthma patients, and randomly 42 SNPs were associated with BDR in population and family-based analyses, among which rs868150 is within the *THRA* gene.

THRB gene polymorphisms

Multiple association studies have been performed on *THRB* gene polymorphisms. The most studied SNPs are reported in figure 1b. These SNPs were related to some diseases and quantitative traits as well as with response to drugs.

Peeters *et al.* (2003) investigated the association of an SNP in exon 10 (rs13081063) of *THRB* gene with plasma TSH and iodothyronine levels, but no significant association was found. Later, Sørensen *et al.* (2008) investigated four SNPs within *THRA* and *THRB* genes to relate them with some thyroid parameters (figure 1b). Of all the SNPs studied, only the polymorphism rs13063628 was associated with an increased level of TSH. Individuals carrying the AA genotype have higher serum TSH level. In this regard, an additional study was carried out by Lopez *et al.* (2008) on a Sardinian population. A genomewide association study was performed, and the SNP rs1505287 ranks among the SNPs that showed strongest association with TSH level, suggesting a possible involvement of *THRB* gene in the variability of TSH levels.

Association studies with *THRB* gene were also conducted for pharmacogenomic purposes. Indeed, a recent study has evaluated the association of SNPs in the transcription factor genes with BDR in asthma trial populations treated with a short-acting β2 agonist. One SNP near the 5′ end of the *THRB* gene (rs892940) was correlated with BDR (Duan *et al.* 2012). Thus, the authors suggested that this investigation identified a novel locus for interindividual variability in BDR and represents a translation of a cellular drug–response study to potential personalization of clinical asthma management.

THRB gene polymorphisms have been included in recent genomewide association studies for different diseases (Giedraitis *et al.* 2009; Carvalho *et al.* 2010). No association was found between some SNPs of *THRB* gene and Alzheimer's disease (Giedraitis *et al.* 2009). However, a positive correlation was reported for rs9833191, rs4858608, rs3951794, rs2196427 with aggressive periodontitis (Carvalho *et al.* 2010). A genomewide association

Table 1. Changes reported in thyroid hormone receptors in diseases.

Alteration type	Cancer	Reference
ARN/protein level	Breast	Zhou-Li *et al*. (1992); Silva *et al*. (2002)
	Hepatocellular	Arbuthnot *et al*. (1989); Lin *et al*. (1995)
	Pituitary	Wang *et al*. (1995); Gittoes *et al*. (1997, 1998)
	Kidney	Puzianowska-Kuznicka *et al*. (2000)
	Thyroid	Wallin *et al*. (1992); Bronnegard *et al*. (1994); Onda *et al*. (2002); Puzianowska-Kuznicka *et al*. (2002)
Mutation	Breast	Dayton *et al*. (1988)
	Hepatocellular	Chan and Privalsky (2006, 2009); Iwasaki *et al*. (2010)
	Pituitary	McCabe *et al*. (1999); Ando *et al*. (2001a, b)
	Kidney	Kamiya *et al*. (2002)
	Thyroid	Bronnegard *et al*. (1994)
	Gastric	Huber-Gieseke *et al*. (1997)
	RTH syndrome	Adams *et al*. (1994); Collingwood *et al*. (1998)
Aberrant splicing	Breast	Silva *et al*. (2002)
	Hepatocellular	Lin *et al*. (1996, 1997); Lin *et al*. (1999)
Methylation	Breast	Li *et al*. (2002); Ling *et al*. (2010)
	Thyroid	Joseph *et al*. (2007); Iwasaki *et al*. (2010)
	Leukaemia	Dunwell *et al*. (2009)
Amplification / gene rearragement	Breast	Van der Vijver *et al*. (1987)
	Gastric	Yokota *et al*. (1988); Wang *et al*. (2002)
	Leukaemia	Dayton *et al*. (1988)
	Breast	Futreal *et al*. (1992, 1994); Chen *et al*. (1994)
Loss of heterozygosity	Lung	Dobrovic *et al*. (1988); 36: Drabkin *et al*. (1988)
	Melanoma	Sisley *et al*. (1993)

study of hematological and biochemical traits in a Japanese population revealed a significant correlation between SNP rs9310736 and two red blood cell traits, minor corpuscular volume and minor corpuscular hemoglobin (Kamatani *et al.* 2010).

Cytogenetic alterations of TRs genes in diseases

The role of TRs in development of human diseases, especially cancer, is well documented (table 1). Indeed, molecular analysis of TR genes allowed identification of some cytogenetic alterations in these genes inducing aberrant gene expression or aberrant activity of their protein products. Table 1 shows the changes reported in TR in some malignancies and in an endocrine disease, resistance to thyroid hormone syndrome (RTH).

Several types of changes in TR were described in cancers while only point mutations were identified in the RTH syndrome. Among the cytogenetic alterations that have been reported in the literature is the epigenetic inactivation of the *THRB* gene through aberrant promoter methylation which was identified in many types of cancer including breast cancer, thyroid cancer and acute lymphoblastic leukaemia (Li *et al.* 2002; Joseph *et al.* 2007; Dunwell *et al.* 2009; Iwasaki *et al.* 2010; Ling *et al.* 2010). Elsewhere, many studies have identified an aberrant alternative splicing of the transcript of *THR*β in their report, Ando *et al.* (2001a) identified a 135-bp deletion within the sixth exon of *THR*β caused by aberrant alternative splicing of *THR*β mRNA in patients with TSH-secreting pituitary tumours. A truncated *THR*β mRNA was also found in patients with breast cancer due to deletion of variable-length truncation ranging from 135 to 705 bp in the sequence encoding the ligand binding domain (LBD) (Silva *et al.* 2002). Also, high frequencies of somatic mutations were observed in many malignancies including hepatocellular cancer (Chan and Privalsky 2006, 2009), pituitary cancer (Ando *et al.* 2001a, b) and renal clear cell carcinoma (Kamiya *et al.* 2002), and in the RTH syndrome (Collingwood *et al.* 1994; Huber *et al.* 2003). The effect of these mutations in some diseases is discussed below.

Mutations in RTH

RTH syndrome is an endocrine disease caused by mutations in the thyroid hormone receptor β that impairs corepressor release in response to T_3 (Jepsen and Rosenfeld 2002; Yen 2003; Rosen and Privalsky 2009). Despite a large number of *TR*β mutations, no RTH mutations have been mapped to TRα in humans. Mutations in the TRβ are generally located in the C-terminal region of the gene (exons 8–10 for *THRB*). In fact, most of the mutations identified are clustered in the LBD and adjacent hinge domain in the protein (figure 2): residues 310–353 (cluster 1), 429–460 (cluster 2) and 234 – 282 (cluster 3). It has been shown that mutations in clusters 1 and 2 impair thyroid hormone binding directly, increase the dissociation of T_3 from its binding site, inhibit the formation of heterodimers, or selectively inhibit coactivator binding (Collingwood *et al.* 1994; Huber *et al.* 2003). However, mutations in cluster 3 affect receptor function indirectly by defective corepressor release (Safer *et al.* 1998).

At least 128 different mutations have been identified in the RTH-TRβ among which 111 mutations are missense/nonsense mutations, six small deletions, six small insertions, two small indel, two gross deletions and one regulatory mutation (Human Gene Mutation Database 2012 available at http://www.hgmd.cf.ac.uk).

Mutations in cancer

It is known that TR mutations can give rise to endocrine disorders. Some TR mutations have been shown to be associated

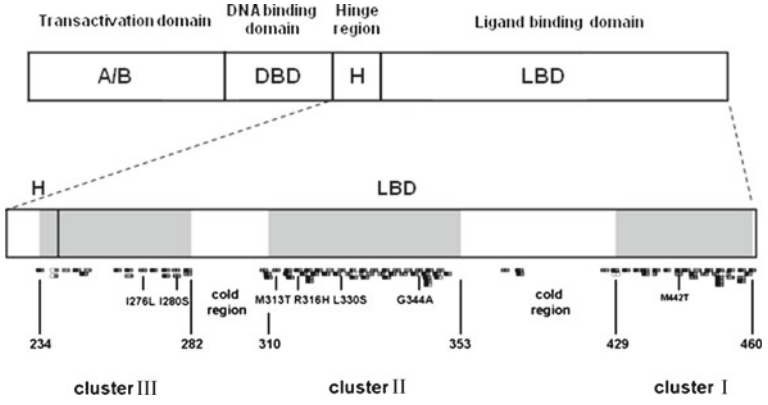

Figure 2. Schematic representation of the thyroid hormone receptor β1 with location of mutations in individuals with RTH. Receptor regions colored in gray indicate hotspot regions of mutations: cluster I (amino acids 429–460), cluster II (310–353) and cluster III (234–282). The location of missense/nonsense mutations is indicated by a symbol with two different colours representing its functional effect: black symbols, reduced T_3-binding affinity; grey symbols, dominant negative effect; white symbols, normal activity; symbols with vertical lines, untested.

with several types of human cancers, including thyroid neoplasia (Puzianowska-Kuznicka *et al.* 2002), renal clear cell carcinoma (RCCC) (Kamiya *et al.* 2002), hepatocellular carcinoma (HCC) (Lin *et al.* 1999) and pituitary tumours (Ando *et al.* 2001b).

Thyroid cancer

The role of TRs in thyroid carcinogenesis was first studied by examination of the expression of TR mRNA in neoplastic human thyroid tissues (Wallin *et al.* 1992; Bronnegard *et al.* 1994). Later, molecular analysis of these receptors revealed presence of mutations in the *THRA* and *THRB* genes. A high prevalence of mutations of *THRA* and *THRB* genes mutations has been identified in a Polish study on papillary thyroid tumours (Puzianowska-Kuznicka *et al.* 2002). In this study, 63% of the thyroid neoplasias were found to have mutations in the *THRA* gene and a remarkable 94% in *THRB* gene, whereas 22% and 11% of thyroid adenomas bore mutations in these genes, respectively. These authors have supposed that these mutated TRs lose their trans-activation function and exhibit dominant negative activity (Puzianowska-Kuznicka *et al.* 2002). These results suggest a critical role for mutated TRs in the tumorigenesis of human papillary thyroid carcinoma. However, more recent studies failed to find this high frequency of TR mutations (Takano *et al.* 2003; Joseph *et al.* 2007; Rocha *et al.* 2007).

Hepatocellular cancer (HCC)

Spontaneous mutations in TRα and TRβ were found at high frequencies in human hepatocellular carcinomas and are believed to have a role in the initiation or progression of this malignancy (Chan and Privalsky 2006). Point mutations in TRα1 and TRβ1 were detected in liver tumours at frequencies of 65% and 76%, respectively. These mutations defined two hotspots for TRα1 in amino acid codons 209–228 and 245–256, while no hotspot was detected in TRβ1 (Lin *et al.* 1999). The TR mutants retained the ability to repress target genes in the absence of T_3 and functioned as dominant negative inhibitors of wildtype TR activity. Yet, several TRα HCC mutations altered the DNA recognition properties of the encoded receptors, indicating that these HCC–TR mutants may regulate a distinct set of target genes from those regulated by wildtype TR (Chan and Privalsky 2006). In fact, it has been shown that the HCC–TR mutants gained the ability to activate several genes known to play proliferative roles (*CSF1*, *NRCAM* and *CX3CR1*) and to repress several genes known to function as tumour suppressors (*DKK1* and *TIMP3*). Conversely, several potential proliferative genes repressed by wildtype TR were not repressed by the HCC–TR mutant (e.g. *GPC3*, expression of which has been linked to cell proliferation in liver), and several potential tumour suppressor genes activated by wildtype TR were not activated by the HCC–TR mutant (e.g., *TIMP3*) (Chan and Privalsky 2009).

Renal clear cell carcinoma (RCCC)

Like HCC, a high TR mutation frequency was found in RCCC. Mutations in TR have been identified at a frequency of 40% in RCCC (Kamiya *et al.* 2002). The majority of the RCCC TR mutants are defective for transcriptional activation and behave as dominant-negative inhibitors of wildtype receptor function. Although several of the dominant-negative RCCC TR mutants are impaired in hormone binding, they failed to liberate corepressors appropriately in response to T_3, a trait that closely correlates with their defective transcriptional properties. Notably, many of these mutants exhibit additional changes in their specificity for different corepressor splice forms that may further contribute to the disease phenotype. Mapping of the relevant mutations reveals that the C-terminal receptor helix 12 is not simply a hormone-operated switch that either permits or prevents all corepressor binding, but is instead a selective gatekeeper that actively discriminates among different forms of corepressor even in the absence of T_3 (Rosen and Privalsky 2009). A recent study, performed by Rosen *et al.* (2011) to determine whether the altered gene recognition observed in HCC was a general phenomenon in the neoplastic phenotype and especially in RCCC, has shown that two different TR mutants (isolated from independent RCCC tumours) possess greatly expanded target gene specificities that extensively overlap with one another, but are clearly different from those of the wildtype TR. Many of the genes targeted by either or both RCCC–TR mutants have been previously implicated in RCCC and include a series of genes that encode metallothioneins and solute carriers, and genes involved in glycolysis and energy metabolism. Thus, the authors suggested that TR mutations from RCCC and HCC may play tissue-specific roles in carcinogenesis, and that the divergent target-gene recognition patterns of TR mutants isolated from the two different types of tumours may arise from different selective pressures during development of RCCC vs HCC (Rosen *et al.* 2011).

Pituitary cancer

TR mutations in pituitary cancer were described in several studies (McCabe *et al.* 1999; Ando *et al.* 2001a,b). In fact, three missense mutations were identified in *THRA* gene in nonfunctioning pituitary tumours; two of these mutations were located in the common region and one of them is TRα2 specific (McCabe *et al.* 1999). Further, two mutations in the LBD of TRβ, which have been reported in the syndrome of RTH were found in TSH-secreting tumours (Ando *et al.* 2001b; Safer *et al.* 2001). These TRβ mutants had impaired T_3 binding and T_3-mediated negative TSH regulation. Yet, the analyses of RT-PCR products of patients with TSH-secreting pituitary tumours revealed a 135-bp deletion within exon 6 that encodes the LBD of TRβ2. This deletion is responsible for the inability of the receptor to bind T_3 and to mediate T_3-dependent negative regulation of TSH

and causes a dominant-negative action over wildtype TRβ2 in transfected cells (Ando *et al.* 2001a).

Conclusion

Thyroid hormone receptors, members of the nuclear receptor superfamily, play a crucial role in many physiological processes. Moreover, increasing evidence shows that these receptors could be involved in development and progression of several types of human diseases. Many studies cited in this review have indicated that cytogenetic alterations of TR genes are closely associated with disorders including cancers, metabolic diseases, neurological diseases and cardiovascular diseases. Other studies have shed light on genetic variation of genes encoding these receptors. These studies have related numerous polymorphic sites of TR genes with the risk of diseases as well as resistance to drug therapy. It is clear that TRs are key proteins in the decipherment of certain disorders, but further investigations are needed to understand the molecular mechanism of action of these genes and use that understanding to predict response to therapy and to develop preventive strategies for the high-risk populations.

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