What is the Role of Thyroid Hormone Receptor Alpha 2 (TRα2) in Human Physiology?

Authors
Sarah Paisdzior[1], Markus Schuelke[2, 3], Heiko Krude[1]

Affiliations
1 Institute of Experimental Pediatric Endocrinology, Charité – Universitätsmedizin Berlin, corporate member of Freie Universität Berlin and Humboldt-Universität zu Berlin, Berlin, Germany
2 NeuroCure Cluster of Excellence; Charité – Universitätsmedizin Berlin, corporate member of Freie Universität Berlin and Humboldt-Universität zu Berlin, Berlin, Germany
3 Department of Neuropediatrics, Charité – Universitätsmedizin Berlin, corporate member of Freie Universität Berlin and Humboldt-Universität zu Berlin, Berlin, Germany

Key words
thyroid hormone receptor alpha, TRα2, mutation, resistance to thyroid hormone, RTH, THRA

received 30.09.2021
revised 19.11.2021
accepted 25.11.2021
published online 07.03.2022

Bibliography
Exp Clin Endocrinol Diabetes 2022; 130: 296–302
DOI 10.1055/a-1716-7980
ISSN 0947-7349

Georg Thieme Verlag KG, Rüdigerstraße 14,
70469 Stuttgart, Germany

Correspondence
Dr. Sarah Paisdzior
Institute of Experimental Pediatric Endocrinology
Charité – Universitätsmedizin Berlin,
Augustenburger Platz 1
13353 Berlin
Germany
Tel.: + 49 30 450 559 828
Sarah.paisdzior@charite.de

ABSTRACT

Thyroid hormone receptors are nuclear receptors that function as transcription factors and are regulated by thyroid hormones. To date, a number of variants and isoforms are known. This review focuses on the thyroid hormone receptor α (TRα), in particular TRα2, an isoform that arises from alternative splicing of the *THRA* mRNA transcript. Unlike the TRα1 isoform, which can bind T3, the TRα2 isoform lacks a ligand-binding domain but still binds to DNA thereby antagonizing the transcriptional activity of TRα1. Although a regulatory role has been proposed, the physiological function of this TRα2 antagonism is still unclear due to limited *in vitro* and mouse model data. Recently, the first patients with resistance to thyroid hormone due to mutations in *THRA*, the TRα encoding gene, affecting the antagonistic function of TRα2 were described, suggesting a significant role of this particular isoform in human physiology.

The thyroid hormones (TH) triiodothyronine (T3) and thyroxine (T4) are important regulators of biological functions. Lack of TH action can lead to developmental, metabolic, and cardiovascular diseases. At the cellular level, nuclear receptors called "thyroid hormone receptors" (THRs) mediate TH function [1]. Heritable syndromes of impaired TH sensitivity include, among others, resistance to thyroid hormone (RTH), which is caused by mutations in genes encoding for THRs, in particular the two genes *THRA* and *THRB* [2]. In general, patients' symptoms depend on the expression pattern of the affected gene and the resulting functional defect. Refetoff et al. coined the acronym RTH when they described the first patient in 1967 [3], who, 20 years later, was found to have a homozygous deletion in the *THRB* gene encoding TRβ [4]. During this time, additional patients with RTH were identified, most of whom carried a heterozygous missense mutation in *THRB* [5, 6]. Patients with RTH have elevated T3 and T4 serum concentrations, with some having elevated, but never suppressed thyrotropin (TSH) levels, making the association with TH-dependent disease relatively clear. The phenotype is variable, and few patients show severe symptoms such as attention-deficit hyperactivity disorder (ADHD), tachycardia, or goiter [7, 8]. TRβ is mainly responsible for the negative feedback loop regulating the hypothalamus-pituitary-thyroid

axis [9, 10], which explains why TSH is not suppressed despite high T3 and T4 levels.

The two genes encoding the TH receptors are TRα (*THRA*) and TRβ (*THRB*), therefore, the differences in the phenotype of patients with TH receptor gene mutations are likely due to different expression patterns of the two isoforms. With the development of new technologies such as whole-exome sequencing (WES), the first *THRA* mutation was identified in 2012 [11], followed shortly by a second case in the same year identified by Sanger sequencing [12]. Since then, the number of *THRA* missense mutations has been steadily increasing. These patients lack the changes in blood TH concentration that make other conditions with RTH so distinct, as the hypothalamus-pituitary-thyroid axis is only regulated by TRβ, whereas TRα plays no role in this feedback loop. Therefore, the T3 and T4 serum levels are mainly normal in these patients. It appears that the T3 levels are in the higher normal range while T4 is rather low-normal resulting in a shifted T3/T4 ratio; however, so far, no reference values are available for the T3/4 ratio leaving the relevance of this shift open. The normal T3 and T4 values in most cases make the phenotype even more striking, as *THRA* mutation carriers have an even more severe phenotype than *TRHB* mutation carriers with mainly hypothyroid symptoms. These include growth retardation, mild to moderate mental retardation, mild skeletal dysplasia, severe constipation, broad facial features, and bradycardia. Interestingly, most of the identified mutations result in partial or complete loss of function that inhibits gene regulation in a dominant-negative manner [11–22].

Recently, we described a novel heterozygous point mutation that enhances the function of both TRα isoforms, TRα1 and TRα2 leading to increased T3-activation of TRα1 and increased antagonism of TRα2 [23]. This novel mechanism in RTH due to *THRA* mutations brings the TRα2 splice variant into focus and raises further interest in its physiological function.

The TRα encoding gene *THRA* (17q21.1) was previously described as a proto-oncogene "c-erb-A" and was isolated from embryonic chicken, human placental, and rat brain libraries [24, 25]. This gene encodes proteins that share structural features with other nuclear receptors, consisting of a regulatory A/B-domain, a DNA-binding domain (DBD), a hinge region, and a ligand-binding domain (LBD) (▶ **Fig. 1a**) [26, 27]. C-erb-A was identified as TRα1 by nuclear localization, ability to specifically bind TH and transcriptional regulation of TH-responsive genes [24, 25]. Briefly, TRα1 can interact with TH responsive elements (TRE) located in the promoter regions of TH-regulated genes *via* the two C_4-zinc fingers in the DBD and regulate transcription (▶ **Fig. 2a**) [28]. Even in an unliganded state, TRs occupy TREs in the function of transcriptional regulators [29].

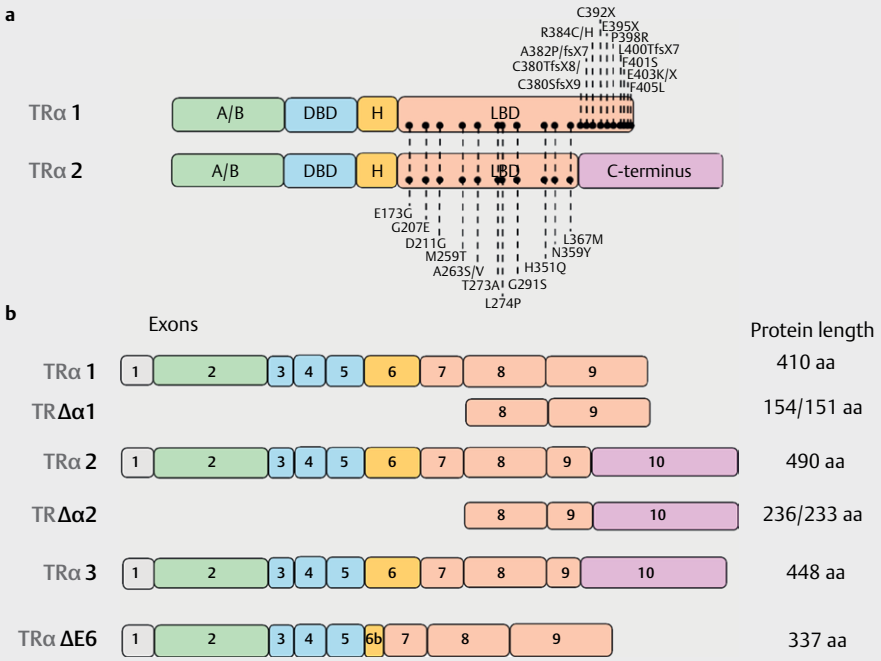
TREs typically consist of a 5´-AGGTCA-3´motif arranged in repeats, either as palindromic, inverted palindromic, or direct repeats spaced by four nucleotides (DR4) [30]. TRs can be positive or negative regulators being able to initiate or inhibit transcription depending on which TRE is bound [31]. The C-terminal LBD is crucial not only for regulating activity through ligand binding but also for interacting with cofactors and dimerizing with other nuclear receptors. Upon binding to T3, the LBD undergoes conformational changes that lead to the replacement of co-repressors (CoRs) by co-activators (CoAs) (▶ **Fig. 2b**) [32]. TRs are known to exist as monomers, but can also form homodimers [33] as well as heterodimers with other nuclear receptors, the most common being the retinoid X receptor (RXR) [34, 35]. Interestingly, RXR has been shown to significantly inhibit transcriptional activity *in vitro*, suggesting a regulatory role for this heterodimer [36].

In addition to the canonical function as transcription regulators, non-canonical TH signaling has a more rapid effect on the target cell. Flamant et al. proposed a classification of TH action into four subtypes [37]: **Type 1** corresponds to the canonical model of TR as a transcription factor by direct binding to DNA, as described above, with TH signaling in mitochondria *via* the shorter isoform (as described below) also belonging to this type. **Type 2** includes signaling *via* indirect binding to DNA, e. g., by binding to other transcription factors. **Type 3** includes signaling independent of DNA binding, such as direct activation of the phosphoinositide 3-kinase/protein kinase B (PI3K/AKT) pathway, which is described in particular for the plasma membrane-bound isoform p30 (see below). **Type 4** summarizes TH signaling independent of TRs, e. g., integrin αVβ3, which has been proposed as a membrane receptor for T3 and T4.

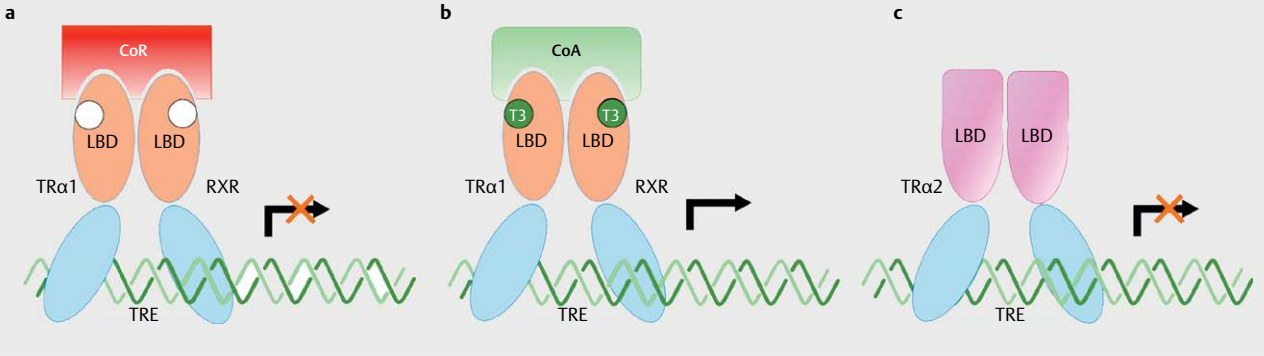
Shortly after the discovery of TRα1, the TRα2- isoform was identified resulting from an alternative splice site at exon 9 and transcription of an additional exon 10 [38, 39] (▶ **Fig. 1b**). Interestingly, this isoform is unable to bind TH due to the extended C-terminal LBD [40] leading to an antagonistic effect on TRα1 and TRβ. However, TRα2 has only a weak antagonistic effect on TRα1 and TRβ, as it needs high expression levels to inhibit transcriptional activity (▶ **Fig. 2c**) [41–44] suggesting a rather minor physiological role for TRα2. Several mechanisms have been suggested to explain this phenomenon: (**i**) competition for TRE binding sites, (**ii**) interaction with RXR as the preferred dimerization partner of TRα1 on specific TREs such as DR4 [45], or (**iii**) a DNA-independent mechanism such as interaction with basal transcriptional factors [41, 46]. Moreover, the phosphorylation state of the elongated C-tail has been shown to influence the antagonistic effect of TRα2 [47]. Interestingly, the inhibitory effect seems to occur only on positive TREs [48], and not on negative TREs. The rather weak effect can be explained by the lack of interaction between TRα2 and CoRs [42, 43].

Nevertheless, protein studies in mouse models and *post mortem* human brains indicate a high expression ratio of TRα2 to TRα1 [49, 50]. This suggests a T3-independent regulatory role for the physiology of TRα2. Important for this role is that TRα2 has a functional DBD, which still binds to TREs as homo- or heterodimer with RXR, albeit with lower affinity as compared to TRα1 [42, 45]. Therefore, as long as TRα2 is highly expressed, it forms homodimers and occupies TREs without the capability to be activated by T3 and thus acts as an antagonist to TRα1. A direct heterodimer with TRα1, which would result in an even more direct TRα1-antagonism, was not observed on any tested TRE. However, since most dimerization studies of TRs examined the formation of dimers on TREs indirectly by using DNA mobility shift assays, it cannot be excluded that heterodimerization between TRα1 and TRα2 may occur independently of DNA-binding, which was suggested by Katz et al. 1995 [47].

Over time, other TRα isoforms were discovered (▶ **Fig. 1b**), including a suspected third splicing variant, TRα3 that originates from another splicing event in exon 9. Similar to TRα2, this isoform presumably has an elongated C-tail encoded by the sequence of exon 10 (448 amino acids), which is also unable to bind to TH. As only one



▶ **Fig. 1** (**a**) Functional domains of TRα1 and TRα2 isoforms. Published disease-causing variants are marked on the protein domain structure. (**b**) TRα isoforms resulting from alternative splicing, or different transcription start points, leading to proteins of different molecular weight. (A/B: a regulatory domain; DBD: DNA-binding domain; LBD: ligand-binding domain)



▶ **Fig. 2** (**a**) The DNA binding domains (DBD) of the unliganded TRα1 homodimer bind to responsive elements of the thyroid hormone receptor (TRE) in the promoter region of target genes. The ligand-binding domains (LBD) are in complex with co-repressors (CoRs) and target gene expression is inhibited. (**b**) Upon binding of thyroid hormone T3, CoRs are exchanged for co-activators (CoA), and the target genes are then expressed. (**c**) The weak antagonistic effect of TRα2 is probably due to competition for TREs between both isoforms without interaction with CoRs.

study was able to detect this isoform, [38] it is vastly understudied, but the predicted structure anticipates the same function as TRα2. Yet another alternative splicing event is responsible for the TRα1-ΔE6 isoform that carries an exchange of exon 6 for the micro-exon 6b. The resulting protein lacks T3-binding capacity but can reduce the transcription-enhancing activity of TRα1. Its proposed role is also regulatory for TRα2 and seems to be important for myocardial development, though the expression pattern of TRα1-ΔE6 suggests additional roles in other tissues [51]. Although it has not been investigated, a TRα2-ΔE6 isoform likely exists as well.

Further truncated isoforms, ΔTRα1 and ΔTRα2, have been observed, which are the result of transcription from an internal promoter in intron 7 and thus are missing the N-terminal domain, but otherwise resemble TRα1 and TRα2 [52]. Additionally, alternative translational start points on the TRα1 mRNA can result in shorter isoforms (p43, p30, p33, and p28) that can be activated by T3 but lack the ability to bind to DNA. They are thought to be bound to the plasma membrane (p30 and p33) [53, 54] or located in the mitochondria (p28 and p43) [55] and maybe responsible for more immediate non-canonical signaling *via* the MAPK pathway.

Since the first description of a loss-of-function *THRA* mutation in 2012, about 26 other missense mutations have been identified. One-half of the identified mutations are positioned in the TRα1-specific region of the LBD [11, 12, 14, 16, 19, 55–60], the other half is located in the coding exons that are shared between TRα1 and TRα2 [13, 15–22, 61] (▶ **Fig. 1**). The latter ones could also disturb the function of TRα2, so the phenotypes seen in these patients might provide information about the physiological role of TRα2.

Although several studies in mice have addressed TRα1 and its function [62–66], the relative contributions of both isoforms to the overall phenotype have proven difficult to dissect. As mentioned earlier, a comparison of protein levels of TR isoforms in mice revealed organ-specific expression, particularly a unique expression pattern of high TRα2 abundance in the central nervous system [49], suggesting an important regulatory role. The specific knockout of TRα2 by adding a strong polyadenylation site that follows the stop codon of TRα1 and transcriptional stop codon resulted in overexpression of TRα1 and a mixed phenotype with hyper- and hypothyroid tissue states [67]. Although this model provides valuable information to the field, it could not fully explain the physiological role of TRα2. Another interesting model is the *Pax8*$^{-/-}$*TRα*$^{0/0}$ compound mouse. Here *Pax8*, a differentiation factor for thyroid cells was knocked out, which on its own results in the absence of thyroid cells and consequently the complete absence of TH. Without T4 treatment, this defect leads to an early death around weaning time [68]. This model was combined with a *TR*$^{0/0}$ model, harboring a complete deletion of all known TRα isoforms, which on its own was viable, but exhibited reduced growth, delayed bone maturation, moderate hyperthermia, and reduced intestinal mucosal thickness [69]. The *Pax8*$^{-/-}$*TRα*$^{0/0}$ compound model survived without TH-treatment and partially rescued the lethal phenotype of *Pax8*$^{-/-}$ mice, but growth was delayed [70]. This study helped to understand how the unliganded receptors might have a physiological function and TH is required to relieve these effects during the postnatal stage. In contrast, *Pax8*$^{-/-}$*TRα1*$^{-/-}$ compound models, which still express TRα2 and ΔTRα2 isoforms, have a similar lethal phenotype to *Pax8*$^{-/-}$ mice, probably due to an intact TRα2 isoform that could affect the activity of other THRs such TRβ [71]. When comparing these two studies, a possible physiological role for TRα2 is to modulate survival, especially in the first weeks of postnatal development.

Most mutations found in patients were studied *in vitro* using reporter gene assays based on TRE-dependent luciferase expression and interaction with DNA or with cofactors to show how they inhibit TRα function. For mutations jointly affecting TRα1 and TRα2, some but not all studies have also examined the effects in both splicing isoforms. However, when TRα2 function was tested, most mutations had no measurable effect on antagonistic function. Interestingly, for two mutations (p.A263S and p.N359Y) the inhibitory effect of TRα2 on TRα1 was slightly reduced [15, 16], suggesting a decrease in the dominant-negative effect.

In contrast to all other studies, we recently reported a *THRA* mutation that resulted in a **gain-of-function** in both isoforms [23]. A mutated glutamate-to-glycine residue in the first helix of the LBD had a promoting effect on T3-inducible TRα1 activity but also resulted in a gain-of-antagonistic effect for TRα2. Based on the computational model of the LBD, we suspect altered dimerization interphase, although an altered interaction with TRE or cofactors cannot be excluded. Nonetheless, this mutation is the first to enhance TRα2 function by increasing its antagonistic capacity, at least *in vitro*. At the same time, TRα1 function was increased as well, leading to a pronounced T3 effect. Given this strong gain of function effect of TRα1 *in vitro*, one would expect a hyperthyroid phenotype of the patients, but this was only the case in patients with mild tachycardia. In fact, we observed more hypothyroid symptoms such as low IQ and global developmental delay, severe constipation, and obesity. Matching these symptoms with our *in vitro* results suggests that the activated antagonistic effect of the mutant TRα2 was able to counteract the increased activity of the mutant TRα1. Since in most brain regions the TRα2: TRα1 ratio is high [49, 50], the mutant TRα2 appears to significantly suppress the activation of the mutant TRα1, eventually leading to the neuronal hypothyroidism of patients with the THRAp.(E173G) mutation. In other tissues with predominant TRα1 expression, such as cardiomyocytes, the gain-of-function mutation of TRα1 without TRα2 antagonism results in hyperthyroidism-like symptoms. These particular findings of the p.(E173G)-mutant, leading simultaneously to activation of TRα1 and enhanced antagonism of TRα2, suggests that the physiological function of TRα2 is antagonistic to TRα1 function, which appears to be important for the tissue-specific fine-tuning of TH action in target cells.

Overall, 33 years after the discovery of TRα2 and almost 10 years after the first description of patients with mutations in *THRA,* the first evidence for a physiological effect of TRα2 was found only recently in particular patients carrying a new TRα-mutation. So far, the finding is limited to a single case report and in principle other -potentially genetic- effects can influence the patient's wide phenotype. However, the obvious antagonistic effect of TRα2, and its increase through this p.(E173G) mutation, proposes a novel mechanism in RTH due to *THRA* mutations and argues that TRα2 indeed plays a role in controlling the local response of target cells to circulating T3. It is tempting to speculate that any mechanism that increases TRα2 expression relative to TRα1 will decrease the cell response to T3. Moreover, even in tissues with low T3 availability, high levels of TRα2, or mechanisms that increase the DNA-binding of TRα2, are more likely to suppress T3-responsive genes. TRα2 was discovered in the 1980s but few publications on this isoform have appeared in recent decades, thus, it is now time to unravel the physiological role of TRα2 at different developmental time points and in different tissues more thoroughly. Here, special attention must be paid to a clear distinction between the isoforms. Most likely, the potential of single-cell sequencing will stimulate this process and could lead to new and surprising discoveries for the other TRα isoforms that have been little studied so far. Unfortunately, the lack of suitable TRα antibodies, let alone isoform-specific antibodies, prevents the generation of genome-wide chromatin immunoprecipitation sequencing data (ChIP-Seq) of any species. For now, a lot of knowledge regarding TRα isoforms remains to be uncovered.

Conflicts of Interest

The authors declare that they had no conflict of interest.

References

[1] Brtko J. Thyroid hormone and thyroid hormone nuclear receptors: History and present state of art. Endocr Regul 2021; 55: 103–119. doi:10.2478/enr-2021-0012

[2] Refetoff S, Bassett JHD, Beck-Peccoz P et al. Classification and proposed nomenclature for inherited defects of thyroid hormone action, cell transport, and metabolism. J Clin Endocrinol Metab 2014; 99: 768–770. doi:10.1210/jc.2013-3393

[3] Refetoff S, DeWind LT, DeGroot LJ. Familial syndrome combining deaf-mutism, stuppled epiphyses, goiter and abnormally high PBI: Possible target organ refractoriness to thyroid hormone. J Clin Endocrinol Metab 1967; 27: 279–294. doi:10.1210/jcem-27-2-279

[4] Takeda K, Sakurai A, DeGroot LJ et al. Recessive inheritance of thyroid hormone resistance caused by complete deletion of the protein-coding region of the thyroid hormone receptor-beta gene. J Clin Endocrinol Metab 1992; 74: 49–55. doi:10.1210/jcem.74.1.1727829

[5] Sakurai A, Takeda K, Ain K et al. Generalized resistance to thyroid hormone associated with a mutation in the ligand-binding domain of the human thyroid hormone receptor beta. Proc Natl Acad Sci 1989; 86: 8977–8981. doi:10.1073/pnas.86.22.8977

[6] Usala SJ, Tennyson GE, Bale AE et al. A base mutation of the C-erbA beta thyroid hormone receptor in a kindred with generalized thyroid hormone resistance. Molecular heterogeneity in two other kindreds. J Clin Invest 1990; 85: 93–100. doi:10.1172/JCI114438

[7] Pappa T, Refetoff S. Resistance to thyroid hormone beta: A focused review. Front Endocrinol 2021; 12: 656551. doi:10.3389/fendo.2021.656551

[8] Ohba K, Sasaki S, Misawa Nakamura H et al. Clinical outcomes of 34 patients with resistance to thyroid hormone beta: A twenty-year experience in Japan. Endocr J 2021. doi:10.1507/endocrj.EJ21-0390

[9] Kakucska I, Rand W, Lechan RM. Thyrotropin-releasing hormone gene expression in the hypothalamic paraventricular nucleus is dependent upon feedback regulation by both triiodothyronine and thyroxine. Endocrinology 1992; 130: 2845–2850. doi:10.1210/endo.130.5.1572297

[10] Dyess EM, Segerson TP, Liposits Z et al. Triiodothyronine exerts direct cell-specific regulation of thyrotropin-releasing hormone gene expression in the hypothalamic paraventricular nucleus. Endocrinology 1988; 123: 2291–2297. doi:10.1210/endo-123-5-2291

[11] Bochukova E, Schoenmakers N, Agostini M et al. A mutation in the thyroid hormone receptor alpha gene. N Engl J Med 2012; 366: 243–249. doi:10.1056/NEJMoa1110296

[12] van Mullem A, van Heerebeek R, Chrysis D et al. Clinical phenotype and mutant TRα1. N Engl J Med 2012; 366: 1451–1453. doi:10.1056/NEJMc1113940

[13] Moran C, Agostini M, Visser WE et al. Resistance to thyroid hormone caused by a mutation in thyroid hormone receptor (TR)α1 and TRα2: Clinical, biochemical, and genetic analyses of three related patients. Lancet Diabetes Endocrinol 2014; 2: 619–626. doi:10.1016/S2213-8587(14)70111-1

[14] Tylki-Szymańska A, Acuna-Hidalgo R, Agostini M et al. Thyroid hormone resistance syndrome due to mutations in the thyroid hormone receptor α gene (THRA). J Med Genet 2015; 52: 312–316. doi:10.1136/jmedgenet-2014-102936

[15] Espiard S, Savagner F, Flamant F et al. A Novel Mutation in THRA gene associated with an atypical phenotype of resistance to thyroid hormone. J Clin Endocrinol Metab 2015; 100: 2841–2848. doi:10.1210/jc.2015-1120

[16] Demir K, van Gucht ALM, Büyükinan M et al. Diverse genotypes and phenotypes of three novel thyroid hormone receptor-α mutations. J Clin Endocrinol Metab 2016; 101: 2945–2954. doi:10.1210/jc.2016-1404

[17] van Gucht ALM, Meima ME, Zwaveling-Soonawala N et al. Resistance to thyroid hormone alpha in an 18-month-old girl: Clinical, therapeutic, and molecular characteristics. Thyroid Off J Am Thyroid Assoc 2016; 26: 338–346. doi:10.1089/thy.2015.0463

[18] van Gucht ALM, Moran C, Meima ME et al. Resistance to thyroid hormone due to heterozygous mutations in thyroid hormone receptor alpha. Curr Top Dev Biol 2017; 125: 337–355. doi:10.1016/bs.ctdb.2017.02.001

[19] Kalikiri MK, Mamidala MP, Rao AN et al. Analysis and functional characterization of sequence variations in ligand binding domain of thyroid hormone receptors in autism spectrum disorder (ASD) patients. Autism Res 2017; 10: 1919–1928. doi:10.1002/aur.1838

[20] Moran C, Agostini M, McGowan A et al. Contrasting phenotypes in resistance to thyroid hormone alpha correlate with divergent properties of thyroid hormone receptor α1 mutant proteins. Thyroid Off J Am Thyroid Assoc 2017; 27: 973–982. doi:10.1089/thy.2017.0157

[21] Korkmaz O, Ozen S, Ozdemir TR et al. A novel thyroid hormone receptor alpha gene mutation, clinic characteristics, and follow-up findings in a patient with thyroid hormone resistance. Hormones 2019; 18: 223–227

[22] le Maire A, Bouhours-Nouet N, Soamalala J et al. Two novel cases of resistance to thyroid hormone due to THRA mutation. Thyroid Off J Am Thyroid Assoc 2020; 30: 1217–1221. doi:10.1089/thy.2019.0602

[23] Paisdzior S, Knierim E, Kleinau G et al. A new mechanism in THRA resistance: The first disease-associated variant leading to an increased inhibitory function of THRA2. Int J Mol Sci 2021; 22: 5338. doi:10.3390/ijms22105338

[24] Sap J, Muñoz A, Damm K et al. The c-erb-A protein is a high-affinity receptor for thyroid hormone. Nature 1986; 324: 635–640. doi:10.1038/324635a0

[25] Weinberger C, Thompson CC, Ong ES et al. The c-erb-A gene encodes a thyroid hormone receptor. Nature 1986; 324: 641–646. doi:10.1038/324641a0

[26] Saatcioglu F, Deng T, Karin M. A novel cis element mediating ligand-independent activation by c-ErbA: Implications for hormonal regulation. Cell 1993; 75: 1095–1105. doi:10.1016/0092-8674(93)90319-L

[27] Ribeiro RCJ, Kushner PJ, Baxter JD. The nuclear hormone receptor gene superfamily. Annu Rev Med 1995; 46: 443–453. doi:10.1146/annurev.med.46.1.443

[28] Dahlman-Wright K, Grandien K, Nilsson S et al. Protein-protein interactions between the DNA-binding domains of nuclear receptors: Influence on DNA-binding. J Steroid Biochem Mol Biol 1993; 45: 239–250. doi:10.1016/0960-0760(93)90338-W

[29] Bernal J, Morte B. Thyroid hormone receptor activity in the absence of ligand: Physiological and developmental implications. Biochim Biophys Acta BBA - Gen Subj 2013; 1830: 3893–3899. doi:10.1016/j.bbagen.2012.04.014

[30] Nelson CC, Hendy SC, Faris JS et al. The effects of P-box substitutions in thyroid hormone receptor on DNA binding specificity. Mol Endocrinol 1994; 8: 829–840. doi:10.1210/mend.8.7.7984145

[31] Näär AM, Boutin J-M, Lipkin SM et al. The orientation and spacing of core DNA-binding motifs dictate selective transcriptional responses to three nuclear receptors. Cell 1991; 65: 1267–1279. doi:10.1016/0092-8674(91)90021-P

[32] Mendoza A, Hollenberg AN. New insights into thyroid hormone action. Pharmacol Ther 2017; 173: 135–145. doi:10.1016/j.pharmthera.2017.02.012

[33] Zubkova I, Subauste JS. Sequences required for the transition from monomeric to homodimeric forms of thyroid hormone receptor α and v-erbA. Mol Cell Endocrinol 2003; 199: 61–72. doi:10.1016/S0303-7207(02)00299-X

[34] Li D, Li T, Wang F et al. Functional evidence for retinoid X receptor (RXR) as a nonsilent partner in the thyroid hormone receptor/RXR heterodimer. Mol Cell Biol 2002; 22: 5782–5792. doi:10.1128/MCB.22.16.5782-5792.2002

[35] Li D, Yamada T, Wang F et al. Novel roles of retinoid X receptor (RXR) and RXR ligand in dynamically modulating the activity of the thyroid hormone receptor/RXR heterodimer. J Biol Chem 2004; 279: 7427–7437. doi:10.1074/jbc.M311596200

[36] Muscat GE, Griggs R, Downes M et al. Characterization of the thyroid hormone response element in the skeletal alpha-actin gene: Negative regulation of T3 receptor binding by the retinoid X receptor. Cell Growth Differ 1993; 4: 269–279

[37] Flamant F, Cheng S-Y, Hollenberg AN et al. Thyroid hormone signaling pathways: Time for a more precise nomenclature. Endocrinology 2017; 158: 2052–2057. doi:10.1210/en.2017-00250

[38] Mitsuhashi T, Tennyson GE, Nikodem VM. Alternative splicing generates messages encoding rat c-erbA proteins that do not bind thyroid hormone. Proc Natl Acad Sci 1988; 85: 5804–5808. doi:10.1073/pnas.85.16.5804

[39] Miyajima N, Horiuchi R, Shibuya Y et al. Two erbA homologs encoding proteins with different T3 binding capacities are transcribed from opposite DNA strands of the same genetic locus. Cell 1989; 57: 31–39. doi:10.1016/0092-8674(89)90169-4

[40] Koenig RJ, Lazar MA, Hodin RA et al. Inhibition of thyroid hormone action by a non-hormone binding c-erbA protein generated by alternative mRNA splicing. Nature 1989; 337: 659–661. doi:10.1038/337659a0

[41] Farsetti A, Lazar J, Phyillaier M et al. Active repression by thyroid hormone receptor splicing variant alpha2 requires secific regulatory elements in the context of native triiodothyronine-regulated gene promoters. Endocrinology 1997; 138: 4705–4712. doi:10.1210/endo.138.11.5541

[42] Tagami T, Kopp P, Johnson W et al. The thyroid hormone receptor variant α2 is a weak antagonist because it is deficient in interactions with nuclear receptor corepressors*. Endocrinology 1998; 139: 2535–2544. doi:10.1210/endo.139.5.6011

[43] Burgos-Trinidad M, Koenig RJ. Dominant negative activity of thyroid hormone receptor variant alpha2 and interaction with nuclear corepressors. Mol Cell Endocrinol 1999; 149: 107–114. doi:10.1016/s0303-7207(98)00253-6

[44] Lazar MA, Hodin RA, Chin WW. Human carboxyl-terminal variant of alpha-type c-erbA inhibits trans-activation by thyroid hormone receptors without binding thyroid hormone. Proc Natl Acad Sci 1989; 86: 7771–7774. doi:10.1073/pnas.86.20.7771

[45] Nagaya T, Jameson JL. Thyroid hormone receptor dimerization is required for dominant negative inhibition by mutations that cause thyroid hormone resistance. J Biol Chem 1993; 268: 15766–15771. doi:10.1016/S0021-9258(18)82321-3

[46] Liu RT, Suzuki S, Miyamoto T et al. The dominant negative effect of thyroid hormone receptor splicing variant alpha 2 does not require binding to a thyroid response element. Mol Endocrinol 1995; 9: 86–95. doi:10.1210/mend.9.1.7760853

[47] Katz D, Reginato MJ, Lazar MA. Functional regulation of thyroid hormone receptor variant TR alpha 2 by phosphorylation. Mol Cell Biol 1995; 15: 2341–2348. doi:10.1128/MCB.15.5.2341

[48] Rentoumis A, Krishna V, Chatterjee K et al. Negative and positive transcriptional regulation by thyroid hormone receptor isoforms. Mol Endocrinol 1990; 4: 1522–1531. doi:10.1210/mend-4-10-1522

[49] Minakhina S, Bansal S, Zhang A et al. A direct comparison of thyroid hormone receptor protein levels in mice provides unexpected insights into thyroid hormone action. Thyroid Off J Am Thyroid Assoc 2020; 30: 1193–1204. doi:10.1089/thy.2019.0763

[50] Visser WE, Swagemakers SMA, Ozgur Z et al. Transcriptional profiling of fibroblasts from patients with mutations in MCT8 and comparative analysis with the human brain transcriptome. Hum Mol Genet 2010; 19: 4189–4200. doi:10.1093/hmg/ddq337

[51] Casas F, Busson M, Grandemange S et al. Characterization of a novel thyroid hormone receptor α variant involved in the regulation of myoblast differentiation. Mol Endocrinol 2006; 20: 749–763. doi:10.1210/me.2005-0074

[52] Plateroti M, Gauthier K, Domon-Dell C et al. Functional interference between thyroid hormone receptor (TR) and natural truncated TR isoforms in the control of intestine development. Mol Cell Biol 2001; Jul; 21(14): 4761–4772. doi: 10.1128/MCB.21.14.4761-4772.2001

[53] Chassande O, Fraichard A, Gauthier K et al. Identification of transcripts initiated from an internal promoter in the c-erbAα locus that encode inhibitors of retinoic acid receptor-α and triiodothyronine receptor activities. Mol Endocrinol 1997; 11: 1278–1290. doi:10.1210/mend.11.9.9972

[54] Kalyanaraman H, Schwappacher R, Joshua J et al. Nongenomic thyroid hormone signaling occurs through a plasma membrane – localized receptor. Sci Signal 2014; 7: ra48. doi:10.1126/scisignal.2004911

[55] Wrutniak-Cabello C, Casas F, Cabello G. Mitochondrial T3 receptor and targets. Mol Cell Endocrinol 2017; 458: 112–120. doi:10.1016/j.mce.2017.01.054

[56] Furman AE, Dumitrescu AM, Refetoff S et al. Early diagnosis and treatment of an infant with a novel thyroid hormone receptor α gene (pC380SfsX9) mutation. Thyroid 2021; 31: 1003–1005. doi:10.1089/thy.2020.0695

[57] Moran C, Schoenmakers N, Agostini M et al. An adult female with resistance to thyroid hormone mediated by defective thyroid hormone receptor α. J Clin Endocrinol Metab 2013; 98: 4254–4261. doi:10.1210/jc.2013-2215

[58] Yuen RKC, Thiruvahindrapuram B, Merico D et al. Whole-genome sequencing of quartet families with autism spectrum disorder. Nat Med 2015; 21: 185–191. doi:10.1038/nm.3792

[59] Sun H, Wu H, Xie R et al. New case of thyroid hormone resistance α caused by a mutation of THRA/TRα1. J Endocr Soc 2019; 3: 665–669. doi:10.1210/js.2019-00011

[60] van Mullem AA, Chrysis D, Eythimiadou A et al. Clinical phenotype of a new type of thyroid hormone resistance caused by a mutation of the TRα1 receptor: Consequences of LT4 treatment. J Clin Endocrinol Metab 2013; 98: 3029–3038. doi:10.1210/jc.2013-1050

[61] Wejaphikul K, van Gucht ALM, Groeneweg S et al. The in vitro functional impairment of thyroid hormone receptor alpha 1 isoform mutants is mainly dictated by reduced ligand sensitivity. Thyroid. 2019; 29: 1834–1842. doi:10.1089/thy.2019.0019

[62] Macchia PE, Takeuchi Y, Kawai T et al. Increased sensitivity to thyroid hormone in mice with complete deficiency of thyroid hormone receptor α. Proc Natl Acad Sci 2001; 98: 349–354. doi:10.1073/pnas.98.1.349

[63] Pilhatsch M, Winter C, Nordström K et al. Increased depressive behaviour in mice harboring the mutant thyroid hormone receptor alpha 1. Behav Brain Res 2010; 214: 187–192. doi:10.1016/j.bbr.2010.05.016

[64] Hönes GS, Rakov H, Logan J et al. Noncanonical thyroid hormone signaling mediates cardiometabolic effects in vivo. Proc Natl Acad Sci 2017; 114: E11323–E11332. doi:10.1073/pnas.1706801115

[65] Bao L, Roediger J, Park S et al. Thyroid hormone receptor alpha mutations lead to epithelial defects in the adult intestine in a mouse model of resistance to thyroid hormone. Thyroid 2019; 29: 439–448. doi:10.1089/thy.2018.0340

[66] Liang Y, Zhao D, Wang R et al. Generation and characterization of a new resistance to thyroid hormone mouse model with thyroid hormone receptor alpha gene mutation. Thyroid 2021; 31: 678–691. doi:10.1089/thy.2019.0733

[67] Saltó C, Kindblom JM, Johansson C et al. Ablation of TRα2 and a concomitant overexpression of α1 yields a mixed hypo- and hyperthyroid phenotype in mice. Mol Endocrinol 2001; 15: 2115–2128. doi:10.1210/mend.15.12.0750

[68] Mansouri A, Chowdhury K, Gruss P. Follicular cells of the thyroid gland require Pax8 gene function. Nat Genet 1998; 19: 87–90. doi:10.1038/ng0598-87

[69] Gauthier K, Plateroti M, Harvey CB et al. Genetic analysis reveals different functions for the products of the thyroid hormone receptor α locus. Mol Cell Biol 2001; 21: 4748–4760. doi:10.1128/MCB.21.14.4748-4760.2001

[70] Flamant F, Poguet A-L, Plateroti M et al. Congenital hypothyroid Pax8−/− mutant mice can be rescued by inactivating the TRα gene. Mol Endocrinol 2002; 16: 24–32. doi:10.1210/mend.16.1.0766

[71] Mittag J, Friedrichsen S, Heuer H et al. Athyroid Pax8−/− mice cannot be rescued by the inactivation of thyroid hormone receptor α1. Endocrinology 2005; 146: 3179–3184. doi:10.1210/en.2005-0114