Thyroide hormone resistance syndromes

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Abstract Thyroid hormone resistance syndromes are a group of genetic conditions characterized by decreased tissue sensitivity to thyroid hormones. Three syndromes are currently recognized, in which resistance to hormone action is due respectively to mutations in the gene encoding for thyroid hormone receptor TRb, impaired T4 and T3 transport, and impaired deiodinase-mediated T4 to T3 conversion. An updated review of each of these forms of resistance is provided, and their pathogenetic mechanisms and clinical approaches are discussed.

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Introduction

Thyroid hormone resistance was first reported in 1967 by Refetoff et al. This is a genetic syndrome characterized by decreased tissue sensitivity to thyroid hormones. The classical form is due in a vast majority of cases to mutations in the thyroid hormone receptor beta (THRB) gene, encoding for one of the two types of T3 nuclear receptor, thyroid receptor β (TRβ). There is also a clinical form of resistance, indistinguishable from the classical form, where no TR mutations exist and whose cause is unknown. Thyroid hormone resistance states have also recently been defined with the presence of mutations of Thyrotropin Receptor (TSHR) gene. Other forms of resistance have also been described, with TSH overproduction and/or decreased TSH receptor sensitivity. All these forms of resistance have been described in both childhood and adulthood. The aim of this review is to provide a comprehensive and updated review of these syndromes, with an emphasis on their pathogenesis, their clinical features and the current and future approaches to their management.
T3 conversion.

and T4 transport and impaired deiodinase-mediated T4 to T3 conversion.

This definition also includes conditions due to impaired T3 synthesis. T3 is the active hormone and binds to nuclear receptors, regulating transcription.

Thyroid hormone resistance due to mutations in the TRb receptor

Mechanism of action of thyroid hormones (Fig. 1)

Thyroid hormones regulate major aspects of the development and metabolism of almost all vertebrate tissues. The thyroid gland secretes to hormone compounds, T4 (thyroxine, 3,5,3',5'-tetraiodo-L-thyronine) and T3 (3,5,3'-triiodo-L-thyronine). T3 also originates in tissue from deiodinase-catalyzed deiodination of T4. In fact, most T3 in the body comes via this route. The entry and exit of thyroid hormones and their metabolites through target cell membranes requires the presence of membrane proteins with a transport function. While there are many transport proteins, monocarboxylate transporter 8 (MCT8) is the most relevant from the pathophysiological viewpoint. This protein is a highly specific transporter of T4 and T3, and plays a significant role not only in the cell action of thyroid hormones, but also in hormone secretion by the thyroid gland (Fig. 1).

The thyroid hormones act by regulating the gene transcription rate: The main action of the thyroid hormones is exerted at cell nucleus level by transcriptional regulation. The thyroid hormones thus control the regional and temporal expression of a large number of genes involved in many physiological processes. T3 is the active hormone at transcriptional level. T4 is essentially a prohormone, although cell membrane actions, generically known as "non-genomic actions" have recently been reported.

A very high number of genes are regulated by the thyroid hormones. Microarray studies comparing tissues from euthyroid and hypothyroid rats and mice in a given target tissue such as the liver may yield 600-1,000 genes whose expression is impaired in hypothyroidism. However, some genes with a particular pathophysiological significance may be emphasized. In regulation of the hypothalamic-pituitary-thyroid axis, hypothalamic TRH and pituitary TSH are negatively regulated by T3. Transcription is increased in the absence of T3, and decreased in the presence of T3. In the heart, thyroid hormones regulate myocardial contractility through control of the transcription of genes such as myosin heavy chains and sarcoplasmic reticulum Ca2+ pump (SERCA). In the liver, thyroid hormones regulate lipogenic enzymes such as the malic enzyme, or cholesterol metabolizing enzymes.

Genomic actions of T3 are exerted through nuclear receptors: As occurs with other hormones, such as steroids or vitamin D, T3 regulates transcription by interacting with proteins located in the nucleus called nuclear receptors. There are two types of T3 receptor in mammals, encoded for by different genes. In humans, the thyroid hormone receptor alpha (THRA) gene in chromosome 17 encodes for the TRα1 receptor and other structurally related proteins, such as TRα2, that do not bind T3; the THRB gene, located in chromosome 3, encodes for receptor proteins TRβ1 and TRβ2 (abbreviated as TRβ). These two proteins are identical except for the amino-terminal end.

Nuclear receptors are transcription factors that may be regulated by the hormonal ligand. They have a dual function. On the one hand, they are able to bind the hormone with high affinity, and are therefore sensitive to the very low levels of free T3 in cells. Hormone or ligand binding to receptor is reversible and occurs in a part of the receptor molecule called the ligand binding domain (LBD). The three-dimensional structure of the LBD consists of a gap or pocket that houses the hormone. In addition to its ligand recognition function, the receptor has, in contrast to other proteins able to bind hormones, an executive function consisting of an ability to interact with DNA and other nuclear proteins. DNA binding occurs through interaction with a specific receptor region known as the DNA binding domain (DBD) which has highly specific sequences called "response elements". In the case of T3, the sequence is known as the thyroid response element (TRE). Other receptor regions, more diffusely distributed over the receptor surface, interact with nuclear proteins having enzyme activities able to repress or activate transcription. These are called "coregulator" proteins and may be "corepressor" or "coactivator" proteins respectively.

Receptors regulate transcription through interaction with other nuclear proteins: To put it simply, in the absence of T3, the receptor is bound to DNA through the DBD and to corepressor proteins, and the gene is silenced. T3 binding
to the LBD does not modify receptor binding to DNA, but introduces a change in the tertiary structure of the receptor by which one of the protein helices (number 12) is folded over the gap housing the hormone. This change results in a loss of binding to corepressors, which dissociate from the complex, and in the exposure of a receptor surface on which the coactivator proteins bind. This scheme occurs with genes which are stimulated by T3, and we then speak of “positive regulation”. There are also many genes having a greater expression in the absence than in the presence of T3, such as the gene encoding for TSH. In such cases, we speak of a “negative regulation”. Although in some cases the mechanism works inversely to positive regulation, i.e. corepressors act as coactivators and conversely, the molecular mechanisms involved are not well known. Positive regulation of TSH by TRH has recently been shown to depend on changes in histone H4, while negative regulation by T3 depends on histone H3. A TRβ with a mutation in a patient with THR blocks the action of T3 on histone H3, preventing negative regulation by the hormone.

T3 receptors are not homogeneously distributed in the different tissues: A very important aspect of the action of the T3 receptors is that the transcriptional activity at molecular and cellular level of all three receptors (TRα1, TRβ1, and TRβ2) is very similar. Although TRβ2 has some specific properties, there are usually only subtle differences between the receptors in terms of their affinity for T3 and their ability to transcribe a given gene. However, great physiological differences exist in the role of each of these isoforms in the body. Such differences result from their tissue distribution. Although all three types of receptor are expressed in most tissues, they are expressed in different proportions. In the liver, TRβ1 accounts for more than 80% of the receptors, while TRα1 predominates in the brain, bone, skeletal muscle, and heart, and TRα2 in the pituitary gland. These receptors mediate T3 action in the above tissues and explain many of the clinical characteristics of patients with THR.
Pathogenetic mechanisms in THR

Changes in the TRβ1 and TRβ2 receptors induce resistance to thyroid hormones: Most cases of thyroid hormone resistance are due to mutations in the THRB gene (Fig. 2) which, as noted above, encodes for TRβ1 and TRβ2. TRα1 mutations in humans have not been discovered yet. Complete deletion of the THRB gene with an absence of TRβ has only been found in the original family reported by Refetoff et al. There are two types of mutation, those with decreasing affinity for T3 and others which, while maintaining a normal affinity for the hormone, cause the receptor to interact in an abnormal way with coregulator nuclear proteins. Pathophysiologically, there is an impaired response of organs expressing TRβ1 and TRβ2. Pituitary sensitivity to T3 decreases as a result of the TRβ2 mutation, and higher circulating T3 levels are therefore required to inhibit TSH. The same occurs at the hypothalamic level for THR regulation. The TRH-TSH-thyroid axis is kept regulated at a higher set point, high T4 and T3 levels coexisting with non-suppressed TSH. In organs such as the liver or kidneys where T3 action is mainly exerted through TRβ1, hormone levels in patients with resistance are adequate to the existing mutation. By contrast, in organs where T3 action is exerted through TRα1, such as the heart and bone, increased T3 levels cause a local state of hyperthyroidism, including tachycardia, arrhythmia, and mineralization disorders respectively.

A same mutation may lead to different clinical forms: Thus, in some cases, even when TRβ1 and TRβ2 have the same mutation, the activity of each receptor is not affected to the same extent and there is a greater resistance at the pituitary level. The consequence is that resistance only occurs at the pituitary level, and the rest of the body has hormone excess. The description of the first cases with these characteristics, i.e. hyperthyroidism with elevated TSH not due to a TSH-secreting tumor, led to the distinction between “generalized resistance” and “pituitary or central resistance” as different conditions. These are not currently considered to be different syndromes, but different forms of clinical expression having a common molecular origin, being extreme manifestations of the same disease. The same mutation may lead to each of these two clinical forms in different patients. As a matter of fact, one of the most complex pathophysiological problems posed by THR is why the same mutation may result in different clinical forms depending on the family or even in patients from the same family.

The reason for increased pituitary resistance in some cases is not known for certain. It may be an extreme case of differences in sensitivity to T3 that the different organs of the same patient may show. In an attempt to clarify the mechanism, the effect of expression in mice of a TRβ mutation that causes central resistance in humans was analyzed. Mice showed elevated TSH and signs of hyperthyroidism, i.e. a resistance apparently limited to the pituitary gland. This would suggest that the abnormal function of the mutated receptor is only apparent in the pituitary gland, while a normal response to T3 is maintained in all other tissues. However, it was noted that the mutated receptor had lost its ability for negative regulation of transcription in all tissues, but kept its regulative ability unchanged, also in all tissues. That is, the effect of mutation was not limited to the pituitary gland, as could be inferred from the mouse phenotype. The problem is that, in the pituitary gland, the loss of negative regulation in the thyrotropic hormone results in increased TSH secretion and subsequent thyroid stimulation. The loss of negative regulation by the mutated receptor in other tissues or organs such as the liver, kidney, muscle, and bone has fewer consequences, with positive regulation being more physiologically relevant. For this reason, the above-mentioned tissues continue to be sensitive to excess thyroid hormones, and symptoms of hyperthyroidism occur.

Mutations are grouped in hot spots in the receptor molecule: One hundred and twenty-four mutations have been reported in approximately 350 families, i.e. the same mutation was present in several families. For example, the R338W mutation has been found in 29 unrelated families. In 95% of cases, the mutations consist of single nucleotide substitutions resulting in changes in a single amino acid, or in a few cases in a termination codon. Other genetic changes in TRβ consist of deletions or insertions with changes in the reading pattern. Most mutations are located in three regions rich in CpG dinucleotides (called CG islands or hot spots) in the carboxyl-terminal region of TRβ, and influence the T3 binding domain and the dimerization domain of the other nuclear proteins (Fig. 2).

The mutated receptor has a negative dominant activity: In principle, it is easily conceivable that a mutation affecting the hormone binding region causes a resistance state, so that decreased receptor affinity means a greater amount of hormone being necessary to produce the same biological effect. The reality is however more complex, because no correlation is found in many cases between receptor affinity for T3 and symptom severity.

In the only reported family with complete recessively inherited TRβ deletion, only homozygous patients had clinical signs, i.e. in heterozygous individuals the product of the other TRβ allele, together with normal TRα, was able to compensate for the lack of one of the TRβ alleles. This does not occur in any other patients with dominantly inherited receptor point mutations, in whom the presence of a single mutated allele is able to cause THR symptoms. In other words, the mutated receptor is able to inhibit the activity of normal receptors; this is the so-called negative dominant action.

Lack of receptors is less harmful than lack of hormones: The negative dominant action of the mutated receptor is due to the property of T3 receptors to exert activity in the absence of ligand. In genes positively regulated by thyroid hormone, the receptor is a potent transcription repressor in the absence of hormone, while in negatively regulated genes, the receptor has transcription induction activity in the absence of hormone. Thus, the lack of hormone is not equivalent to the lack of receptor, but is much more harmful, as we have shown in experimental animals. In mice with TRα1 deletion, experimental hypothyroidism induces less changes than in normal mice. In mice also, TRβ deletion causes THR. However, TRβ1 mutation also causes severe structural and functional cerebellar changes due to the negative dominant activity of the mutated receptor.

The binding of the mutated receptor to DNA is required for negative dominant activity: One of the most potent
mutated receptors, the one isolated from family S\(^{20,21}\) (threonine 332 deletion), is able to inhibit even transcriptional response to retinoic acid and vitamin D\(^2\). Negative dominant action is possible because DNA binding is intact. In fact, the introduction of mutations in this area does not induce negative dominant activity or suppresses it in a previously mutated receptor\(^2\). For negative dominant activity to occur, dimerization domains should also be intact. The mutated receptor would thus be able to form heterodimers on DNA, causing repression even in the presence of hormone and interfering with the activation of normal receptors mediated by T3. Lack of T3 binding would cause the receptor to be permanently forming a repressor complex.

Some mutations alter receptor interaction with coactivator or corepressor nuclear proteins: In most cases of HTR, negative dominant activity occurs because lack of T3 binding to the receptor prevents the release of corepressors and the binding of coactivators. There are, however, cases where T3 binding to the mutated receptor is not impaired. In such cases, resistance is due to defective interaction of the mutated receptor with corepressors or coactivators. In this regard, mutations conferring on the receptor different properties have been reported: 1) there are mutations conferring on the receptor an increased affinity for corepressors\(^2\), 2) other mutations are characterized by causing a slow dissociation from the corepressor; 3) the third group of mutations cause defective binding of coactivators\(^2\); 4) finally, some mutations selectively modify interaction with some coactivators, but not with others, introducing heterogeneity in clinical presentations depending on the tissue distribution of the different coactivators. The case reported by Wu et al\(^2\) is of great interest because it illustrates how a mutation may change not only the selectivity of interaction with the receptor but also with the coregulatory molecules. The clinical characteristics of the resulting syndrome will greatly depend on the differential expression of these molecules in different tissues. In the abovementioned case, the syndrome almost only consisted of pituitary signs. The great variety of potential clinical presentation forms may easily be inferred.

Non-TR resistance syndrome: Receptor mutations could not be shown in approximately 15% of patients with resistance. These are known as non-TR resistances and were first reported by Weiss et al\(^2\). It is suspected that these cases may have mutations in some of the many components directly or indirectly interacting with the receptor and which are part of the aggregate complex of coregulatory proteins which eventually interact with the transcriptional machinery. Immediate attention has been directed to searching for mutations in coressor or coactivator proteins. In mice, deletion of the SRC-1 coactivator causes a thyroid hormone resistance state\(^2\). However, efforts to show changes in corepressors or coactivators in humans have been unsuccessful to date\(^2\). The possibility of mosaicism, i.e. when the mutated receptor is not present in all tissues\(^2\) and the detection of mutations depends on whether or not it is present in blood cells, should be considered. However, this possibility has been ruled out in many cases using linkage analysis\(^2\).

### Clinical characteristics

THR is an uncommon autosomal genetic disease: THR is a familial disease, but approximately 28% of mutations occur de novo. A little over 600 cases have been reported in 350 families, with a probable incidence of 1/50,000. In a Japanese study conducted on 83,232 newborns using neonatal screening data, elevated T4 levels were found in 11 infants. These included one case of familial dysalbuminemic hyperthyroxinemia, two of THR, and eight of neonatal Graves disease\(^1\). The original case reported by Refetoff et al\(^1\), due to TR\(\beta\) deletion, was recessively inherited, with the syndrome occurring in homozygous individuals only. This is the only reported case of THR due to TR\(\beta\) deletion. All subsequently reported cases were dominant in nature. One patient in whom the mutation was present in both alleles experienced an extremely severe form of resistance\(^1\). A case of mosaicism, in which mutation was only present in some tissues, was recently reported\(^2\).

Signs of deficiency and hormone excess coexist in THR: A comprehensive description of clinical characteristics was published some years ago in a very extensive review\(^2\). More recent descriptions may also be consulted\(^2,3,31\). The essential finding in these patients is the presence of high circulating thyroid hormone levels (free T4 and T3) concomitant with normal or slightly elevated TSH levels. As noted above, this is due to a decreased sensitivity of the pituitary thyrotropic cell to the action of T3 in inhibiting TSH production, as well as a lower threshold of the neurons of the hypothalamic paraventricular nucleus in TRH production. Increased TSH secretion causes thyroid gland stimulation, of which goiter, occurring in 95% of patients, is the most common sign. Increased thyroid hormone secretion causes hyperthyroidism in patients with limited resistance to the pituitary gland. Patients with generalized THR may be maintained in a euthyroid state. However, clinical signs consistent with hormone deficiency or excess affecting the heart, central nervous system, and development are commonly found. Tachycardia, due to the action of excess hormone through TR\(\alpha\)1, present in the heart, is found in a high number of patients. Some patients, even with a normal heart rate, have symptoms similar to those of hyperthyroidism\(^2\). Bone mineral density is normal in the spine of adult individuals, but is significantly decreased when measured in the femoral neck, and osteoporosis may therefore be a significant problem in THR. Biopsy studies suggest a lower bone formation rate.

Signs of impaired development include low height, delayed dentition and bone age, and deafness. As regards the central nervous system, resistance syndrome in children is often associated with learning disorders, backwardness at school, speech disorders, and even mental deficiency with a low intelligence quotient in 5%-10% of cases. Seventy percent of children with THR have attention deficit hyperactivity disorder (ADHD). This syndrome also occurs in 50% of adults with THR. These rates are higher than those reported in the general population (20% and 7% respectively), and it has therefore been suggested that ADHD syndrome could be genetically linked to mutations in the T3 receptor. There is however no increased incidence of THR in subjects with ADHD as compared to the general population. The
association of THR with a low intelligence quotient could increase the probability of ADHD symptoms\textsuperscript{35}.

**Diagnosis**

THR syndrome should be suspected in individuals with high serum thyroid hormone levels together with normal or elevated TSH levels, particularly when there is goiter. Serum levels of both total and free T4 and T3 are elevated. The minimum requirement for diagnosis is an actual elevation of free T4 in the presence of non-suppressed TSH. Differential diagnosis should be made with hyperthyroidism due to Graves disease or multinodular adenoma, a TSH-secreting pituitary adenoma, and abnormalities in thyroid hormone transport proteins.

Because of the presence of goiter with some signs of hyperthyroidism, such as tachycardia and occasionally hyperactivity, diagnosis of Graves disease is relatively common, as is the use of aggressive treatments such as partial thyroidectomy, obviously with no result because goiter also recurs after a short time. The detection of unsuppressed basal TSH levels in THR and the presence of circulating antibodies in Graves disease may help in establishing diagnosis. However, THR and Graves disease may coexist in the same patient\textsuperscript{36}. These patients require high thyroid hormone doses to maintain euthyroidism after thyroid ablation. In TSH-secreting adenomas there are increased levels of the TSH alpha subunit, and the diagnosis of adenoma is made following X-ray examination.

Changes in transport proteins may be confused with THR: Changes in transport proteins may result in increased total T4 serum levels in the presence of normal TSH levels. Familial dysalbuminemic hyperthyroxinemia\textsuperscript{37}, due to the presence of circulating albumin with a high affinity for T4, should particularly be considered. Under normal conditions, albumin transports up to 10\% of serum T4, but with dysalbuminemic disorders it may transport up to 30\%. Total T4 is elevated, but free T4 remains normal. Changes in transthyretin, erroneously diagnosed as hyperthyroidism or THR, have also been reported\textsuperscript{38}. Changes in transport proteins are not usually associated with symptoms, and a selective increase in total T4 usually occurs, while both free T4 and total and free T3 remain within normal limits. There may be equivocal cases where the presence of goiter due to other causes and an uncertain hormone profile may lead to diagnostic problems. This is particularly true if the free T4 fraction is measured by methods using a T4 analogue because the results are abnormally high due to the presence of abnormal transport proteins. Special procedures, such as free hormone measurement using equilibrium dialysis and serum electrophoresis following incubation with labeled T4, are required in these cases. However, these measurements are not usually available to the clinical laboratory, and THR may therefore be ruled out by carefully monitoring total T3 levels, which are usually normal, or by testing TRH after treatment with T3 as described in the following paragraph.

Response to T3 administration is reduced in patients with THR: Clinical diagnosis of THR is established by assessing the response to T3 administration using the protocol reported by Refetoff et al\textsuperscript{32}. This protocol consists of the administration of escalating daily doses of T3 in three one-week periods (50, 100, and 200 µg/day in adults) in two divided doses. These doses are adjusted for children based on age and weight. A 100 µg adult dose is equivalent to 25 µg in children weighing 8-15 kg (1-3 years), to 50 µg in children weighing 16-25 kg (4-9 years), and to 75 µg in children weighing 26-45 kg (10-14 years). A TRH test is performed before treatment and at the end of each week. In normal individuals, the 50 µg dose of T3 is sufficient to decrease the response to almost total suppression. Much higher doses are required in patients with THR, and TSH increases may be achieved with the highest dose\textsuperscript{39}. If, in addition to TSH, other T3 action parameters such as cholesterol, ferritin, creatine phosphokinase, hydroxyproline excretion, or sex hormone binding globulin (SHBG) levels are measured, it may be noted that while normal subjects already respond to the lower T3 dose, high doses are needed and much lower responses are achieved in patients with THR. Final diagnosis may be confirmed by TR\(b\) sequencing. Since TR\(b\) may easily be sequenced, this is a practical test which can be performed prior to implementing the Refetoff et al protocol, which in fact may be unnecessary if a mutation is detected, especially if such a mutation has previously been reported to be pathogenetic. The pathogenetic nature of a given mutation can be easily demonstrated at a specialized laboratory by analyzing its activity on cell lines. It should be noted that 15\% of patients have no receptor mutations, so that while the identification of mutations confirms resistance, their absence does not rule it out.

Although no treatment is required in most patients, some special situations should be considered: Patients with THR in a compensated euthyroid state do not need treatment, and interventions tending to decrease circulating hormones should be avoided\textsuperscript{40}. While goiter is of moderate severity in most cases, it may sometimes require treatment because of its size. Surgery should not be performed. Good results have been achieved by administering high T3 doses on alternate days\textsuperscript{39}. In patients with signs of tissue hypothyroidism, such as delayed development in children or hypercholesterolemia in adults, treatment with T4 is required, even at high doses, using normalization of TSH levels and other indicators of peripheral action as evidence of biological activity\textsuperscript{40}. Tachycardia should be treated with beta-adrenergic blockers such as atenolol. Patients with resistance restricted to the pituitary gland, with signs of peripheral hyperthyroidism, pose special problems. Circulating thyroid hormone levels should be decreased in these patients without causing chronic TSH elevation due to the risk of inducing thyrotropic cell hyperplasia. Dopaminergic agents are helpful, but lose their effectiveness when chronically administered. T3 acetic derivative, TRIAC (3,5,3'-triiodo-thyroacetic acid), has been used in some cases with some effectiveness\textsuperscript{40,41} because it exerts a greater inhibition of TSH secretion than the stimulation of peripheral metabolism.

The management of THR patients during pregnancy poses special problems: Depending on whether or not the mutation is present in the fetus, a decreased viability of normal fetuses or a lower birth weight have been seen, probably due to excess maternal hormone\textsuperscript{42}. Fetuses with the same mutation as the mother, but not normal fetuses, would be protected from hormone excess. Mothers with asymptomatic
THR would not require treatment\(^4\). However, determination of fetal genotype has been suggested. If the mutation is also present in the fetus, treatment is not required. In the event of a normal fetus, it has been advised to keep maternal free T4 levels under 20\% of the upper normal limit using closely controlled treatment with antithyroid agents\(^5\). Special care should of course be taken not to induce fetal hypothyroidism.

**Impaired cell transport of thyroid hormones**

Mutations in the thyroid hormone transporter MCT8 cause severe neurological changes: In 2004, two groups independently reported in several families a neurological syndrome and mental retardation in children linked to chromosome X\(^4\). The neurological condition consisted of psychomotor retardation, profound mental retardation, lack of verbal communication, axial hypotonia, and deficient control of head posture combined with limb spasticity eventually leading to tetraplegia. Patients had changes in circulating thyroid hormone levels consisting of decreased T4 and rT3 levels and increased T3 levels. TSH was normal or slightly elevated. The hormonal picture suggested an impaired distribution and metabolism of thyroid hormones, and mutations were found in a protein called MCT8, encoded by gene SLC16A2 (solute carrier family 16 member 2, at locus Xq21), whose known role is to transport T4 and T3 through the cell membrane\(^6\).

One year later, in 2005, Schwartz et al\(^7\) found mutations in MCT8 in patients from several families with an X-linked neurological and mental retardation syndrome already independently reported in several families a neurological syndrome and mental retardation in children linked to chromosome X\(^4\),\(^4\). The neurological condition consisted of psychomotor retardation, profound mental retardation, lack of verbal communication, axial hypotonia, and deficient control of head posture combined with limb spasticity eventually leading to tetraplegia. Patients had changes in circulating thyroid hormone levels consisting of decreased T4 and rT3 levels and increased T3 levels. TSH was normal or slightly elevated. The hormonal picture suggested an impaired distribution and metabolism of thyroid hormones, and mutations were found in a protein called MCT8, encoded by gene SLC16A2 (solute carrier family 16 member 2, at locus Xq21), whose known role is to transport T4 and T3 through the cell membrane\(^6\).

**Clinical characteristics**

The syndrome is characterized by an endocrine and a neurological component, which are clearly differentiated\(^8\),\(^9\). The circulating thyroid hormone profile is highly characteristic and unusual, with T3 elevation and T4 and rT3 decrease. TSH may be slightly elevated, but in most cases is normal or in the upper limit of normal and has little diagnostic value. X-linked neurological damage\(^10\), mental retardation, and delayed development are seen, in addition to endocrine changes. These, therefore, occur in children, and women are carriers. A single case of neurological involvement in a woman has been reported. This was probably due to biased inactivation of chromosome X\(^11\).

Patients appear normal at birth. In one of the cases, T4 was slightly decreased at neonatal screening, but TSH was normal. In most cases, the syndrome starts to become evident in the first few months of life as hypotonia of the trunk and an inability to hold the head, sit down, or crawl. Hypotonia occurs in 100\% of cases. There is an overall developmental delay and an almost total lack of language acquisition. Hypotonia progresses to spasticity over time. Mental retardation is profound and, in fact, intelligence quotient remains under 30\(^12\). Other signs include abnormal hand posture, elongated facies and asthenic body build, and wasting due to peripheral hyperthyroidism. Abnormal movements consisting of limb extension on one side and simultaneous contralateral flexion with head rotation are common in children and may dominate the clinical picture\(^13\), while infants may experience episodes of paroxysmal dyskinesia provoked by any stimulus\(^14\).

**Pathophysiology**

In order to study the pathophysiology of this syndrome, mice have been genetically modified by deleting one of the gene exons, which results in the production of a non-functional protein\(^15\),\(^16\). Mice show the endocrine picture, but unfortunately do not experience neurological changes. Thus, they are only a partial model of the disease.

The following conclusions have been drawn from the study of these mice: Mct8\(^*\) is an essential protein for T3 transport to the brain, mainly because of its location in the blood-brain barrier\(^17\),\(^18\). In the absence of Mct8, T3 does not arrive in adequate amounts to the brain (Fig. 3). Although Mct8 also transports T4, this may also cross the barrier by means of another transporter, organic anion transporter polypeptide 14 (Oatp14), a product of the gene solute carrier organic anion transporter family 1c1 or Slc1c1. Mct8 is also expressed in thyroid cells and is involved in T4 and T3 secretion\(^19\),\(^20\). In the absence of Mct8, T4 and T3 accumulate in the thyroid gland, with decreased T4 secretion and possibly (although this is still controversial) increased T3 secretion by mechanisms not clearly known. Mct8 knockout mice also show a marked increase in deiodinase 1 (D1) activity in the liver and kidneys. Mice usually have cerebral hypothyroidism, though partially compensated, and peripheral hyperthyroidism, which causes the wasting experienced by patients.

Several processes contribute to the increase in circulating T3 levels (Fig. 4), including a potential increase in thyroid secretion of T3, which is still controversial\(^21\),\(^22\), and a lower degradation by deiodinase 3 (D3) in organs such as the brain. In addition, higher circulating T3 levels increase D1 activity in both the liver and kidney. T4 to T3 conversion is therefore increased, contributing to a further increase in circulating T3 levels. Decreased circulating T4 levels are due to reduced T4 secretion and to increased T4 to T3 conversion in the liver and kidney. Since deiodinase 2 (D2) is negatively regulated by T4, a decrease in T4 causes an increase in its activity in the brain, with a resultant increase in T4 to T3 conversion. This latter effect compensates, at least partially, for cerebral T3 deficiency in experimental animals. Decreased levels of rT3 are explained by its increased degradation due to the increase in D1 activity in the liver and kidney, and by its decreased production from T4 in the brain.

The pathogenesis of neurological involvement is unknown, and no data are available about the time during fetal and postnatal development when neurological damage starts to occur or which cell lines are affected and which signaling
pathways are involved. The lack of neurological changes in Mct8 knockout mice has prevented detailed research on the syndrome. Compensation of cerebral hypothyroidism occurs in mice through a D2-dependent mechanism that increases T3 supply from T4 in the brain (Fig. 3). Animals have no signs of cerebral hypothyroidism, and only some of the T3 target genes are altered. Magnetic resonance imaging has shown delayed myelination in patients, which could possibly explain neurological deficits. This hypothesis is also supported by the observation that the condition is similar to Pelizaeus-Merzbacher, as noted above.

A hypothesis which could explain the differences between mice and humans is that in the former, the presence of other transporters such as Oatp14 or Lat-2 in the blood-brain barrier allows for the passage of T4 into the brain and T3 formation (Fig. 3), while in the fetal human brain both T3 and T4 passage depend on MCT8 only. This hypothesis is supported by a very recent study showing that the blood-brain barrier of monkeys does not contain OATP1C1, the equivalent to Oatp14 in mice.

Diagnosis

Serum levels of total and free T4 and T3 should be measured in any patient with a history of neonatal hypotonia and cognitive deficiency. Increased T3 levels, particularly if associated with T4 decrease, are almost pathognomonic. Diagnosis is confirmed by gene sequencing.

Treatment

There is currently no effective treatment, but some therapeutic measures have been tested. Since patients have low serum thyroxine levels, treatment with T4 was tested with no clinical improvement, and even with a worsening of peripheral hyperthyroidism. To prevent the latter complication, propylthiouracil was administered to block thyroid function, administering T4 until normalization of T4, T3, and TSH levels. No neurological improvement was achieved, but the general condition of the patient markedly improved. A diiodothyronine, DITPA (3,5-diiodothyropropionic acid), is currently being tested. This molecule is an agonist of the T3 nuclear receptor, to which it binds with low affinity, and has cardiac activity. In fact, it is being tested in Phase II clinical trials for the treatment of heart failure. DITPA crosses the blood-brain barrier in mice, even in the absence of Mct8, and has a minimum metabolic activity. The treatment of patients with this analogue has been effective for improving general but not neurological impairment.

Deiodination defect

The first genetic defect of thyroid hormone metabolism was reported in 2005 by the Refetoff et al group. A study of thyroid function in two families with a low height and delayed bone development found a circulating thyroid hormone profile consisting of elevated T4 and T3 levels and decreased T3 levels, just the opposite to that reported in the previous section on MCT8 mutations. A biochemical and molecular study using patient fibroblasts led to the conclusion that the origin of the syndrome was a defect in...
Thyroid hormone resistance syndromes are not currently limited to changes in the nuclear action of T3, which are the most widely known. When abnormal T4, T3, and TSH levels that cannot easily be explained are found, the possibility of changes in deiodinase transport and synthesis such as those reported, whose actual incidence is not yet known, should be considered. As regards MCT8 mutations, it should be borne in mind that patients will first attend pediatric neurology departments, which may not be familiar with thyroid pathophysiology. These patients will tend to be diagnosed with a congenital hypotonia of unknown cause.

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