Mutations of the Human Thyrotropin Receptor Gene Causing Thyroid Hypoplasia and Persistent Congenital Hypothyroidism*

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ABSTRACT

The pathogenesis of congenital hypothyroidism due to thyroid dysgenesis is still unknown. A point mutation in the TSH receptor (TSHR) of the hypothyroid hty/hty mouse invoked the TSHR as a candidate gene for congenital hypothyroidism. Therefore, we screened for mutations in the TSHR gene in patients with congenital hypothyroidism and hypoplasia of the gland. In one girl detected in neonatal screening with the confirmed diagnosis of permanent congenital hypothyroidism with reduced thyroid volume, two novel mutations in the TSHR gene were identified. Single strand conformational polymorphism and subsequent DNA sequencing studies of a fragment of the TSHR gene showed that the patient is a compound heterozygote for 2 loss of function mutations in exon 10 of the TSHR gene. In the mutant maternal allele, 18 nucleotides (positions 1217–1234) are deleted, and 4 novel bp are inserted, resulting in a frameshift and premature termination of the coding sequence. Transfection studies showed that this truncated TSHR was trapped intracellularly and completely lacked cell surface expression. The paternal gene harbors a missense mutation at nucleotide position 1170, leading to the exchange of the highly conserved C-390 for a W residue. This alteration resulted in a drastic loss of affinity and potency of TSH acting at the mutant compared to the wild-type receptor. In contrast to the published loss of function mutations of the TSHR leading to euthyroid hyperthyrotropinemia, the two new mutations lead to persistent congenital hypothyroidism and defective organ development. Further studies will have to analyze to what extent TSHR mutations are involved in the pathogenesis of congenital hypothyroidism as opposed to other genetic or environmental factors. (J Clin Endocrinol Metab 82: 3471–3480, 1997)

CONGENITAL hypothyroidism (CH) is the most frequent congenital defect of the endocrine system, characterized by a reduced biological impact of thyroid hormone on metabolic processes throughout the body. In the vast majority of cases, the diminished secretion of thyroid hormone results from defective thyroid development, i.e. thyroid dysgenesis, ranging from hypoplasia and ectopy to athyrosis (1). The pathological mechanisms leading to persistent CH are still poorly understood. The pituitary glycoprotein TSH is the major regulator of growth and differentiation of the thyroid cell (2). TSH exerts its cellular effects by binding to and activating a membranous receptor that belongs to the large superfamily of heptahelical G protein-coupled receptors (2–4). The initiation of expression of the TSH receptor (TSHR) on day 14 of mouse embryogenesis (5) indicates that loss of function mutations of the TSHR gene could result in defective thyroid development.

The hypothesis that the TSHR gene is a candidate gene for thyroid dysgenesis was strengthened by the finding that a homozygous loss of function mutation in a highly conserved P in the fourth transmembrane domain of the TSHR causes hypothyroidism and thyroid hypoplasia in the hty/hty mouse (6, 7). The first somatic and germline mutations in the coding sequence of the human TSHR have been introduced as pathogenic entities causing gain of function syndromes (8). Recently, two different missense mutations in the large extracellular N-terminal domain of the human TSHR were identified (9) (Fig. 1). In contrast to patients with congenital hypothyroidism, the three affected compound heterozygote siblings were euthyroid and had normally sized thyroid glands, probably due to the capability of the markedly elevated TSH level to compensate for the receptor defect.

In the present report we describe the first case of thyroid hypoplasia and persistent congenital hypothyroidism due to two novel loss of function mutations in exon 10 of the TSHR gene. The patient is a compound heterozygote, having inherited distinct defective alleles from both father and mother. The mutant maternal TSHR gene encodes a truncated receptor without biological activity. The paternal gene harbors a missense mutation in the coding sequence for a C residue that is highly conserved among glycoprotein hormone receptors (3). This mutation results in severely impaired receptor activity.

Subjects and Methods

The patient

The patient was the first of two daughters born at term to nonconsanguineous parents. At the time of neonatal screening the girl had a
blood TSH concentration of 89 mU/L (normal, <20 mU/L). At reex-
amination, a serum TSH concentration of 82 mU/L in conjunction with
decreased serum concentrations of T4 (78 nmol/L), free serum T4 (FT4;
8.2 pmol/L), and serum T3 (1.4 nmol/L; Table 1) were detected. The
constellation of elevated TSH levels combined with below normal FT4
concentrations was considered diagnostic of congenital primary hypo-
thyroidism. Ultrasound examinations of the newborn revealed a small
thyroid gland with a reduced volume (<0.5 mL) compared to normal
values (0.7–2.4 mL) obtained from healthy newborns in the same moder-
ately iodine-deficient geographic area (10). The transplacental passage
of maternal antithyroid antibodies was ruled out by negative tests for
antithyroid peroxidase, antithyroglobulin, and TSH binding inhibitory
antibodies and antibody-dependent cell-mediated cytotoxicity in the
serum of the newborn. There was no history of excess perinatal iodine
contamination, and the iodine concentration in a randomly chosen urine
sample of the newborn was normal (85 μg/L). The clinical data collected
prompted the initiation of T4 supplementation therapy with a dose of 50
μg l-T4/day (13 μg/kg BW). Discontinuation of T4 treatment for 4 weeks
at the age of 2 yr resulted in a marked increase in the serum TSH
concentration (79 mU/L) concomitant with decreased T4, FT4, and T3
concentrations, thus confirming the diagnosis of persistent congenital
hypothyroidism. Ultrasonography again revealed a reduced volume of
the thyroid gland compared with values obtained from an age-matched
normal population (Table 1). Consequently, l-T4 therapy was reinstalled
and has not been discontinued since then. Linear growth of the presently
5-yr-old girl was normal along the 75th percentile, and determinations
of bone age at 2 and 4 yr of life showed no advancement with respect
to chronological age. The girl’s mental development, as studied at the
age of 5 yr, applying the Hamburg Wechsler intelligence test (11), was
normal, and no neurological abnormalities were observed.

Assessment of thyroid function and thyroid size

Serum concentrations of T4 and T3 were measured with an immuno-
fluorometric assay (Delfia, Wallac, Freiburg, Germany). Serum free T4,
antithyroid peroxidase, and antithyroglobulin antibodies were mea-
sured by RIA (Dynotest, Brahms Diagnostika, Berlin, Germany). TSH
binding inhibitory antibodies were measured by TRAK assays (Brahms
Diagnostika, Berlin, Germany). Antibody-dependent cell-mediated cy-
totoxicity was assessed using a chromium release assay in human thy-
roid cells (12). For ultrasonographic examinations, a 7.5-megahertz
transducer (Acuson 128, Computer Sonography, Berlin, Germany) was
used.

Isolation and characterization of genomic DNA encoding
the TSHR

Genomic DNA was isolated from peripheral blood leukocytes from
selected family members with the help of a DNA extraction kit (QIAamp
Blood Kit, Qiagen, Hilden, Germany). Exons 1–10 of the TSHR gene were
amplified by PCR techniques as described previously (13). In addition,
specific primer pairs were designed to amplify exon 10 of the TSHR gene
agarose gels and by dideoxy sequencing (15). Products were further characterized by restriction endonuclease digestion (SSCP) analysis was carried out as described by Orita et al. (9).

Cell culture, transfection, and functional assays

COS-7 and CHO-K1 cells were cultured and transfected as described previously (18). To establish CHO-K1 cell lines permanently expressing wild-type and mutant TSHRs, the complementary DNA (cDNA) coding for the human TSHR previously (18, 19). To establish CHO-K1 cell lines permanently expressing wild-type and mutant TSHRs, receptor constructs and the plasmid pCD-P5 (16). Novel mutant TSHRs detected in this study were created by employing standard PCR-based mutagenesis techniques (17). Thus, the PCR fragment containing the paternal mutation (TSHR-C390W) was digested with MscI/Bsu36I, the fragment containing the maternal mutant (TSH-R-419trunc) was cut with BsaAI followed by separation of resulting fragments on 2% agarose gels and by dyeoxy sequencing (15).

Radioligand binding assay

Binding studies were performed using membrane homogenates prepared as described previously (20). Incubation buffer consisted of 50 mmol/L Tris (pH 7.4), 3 mmol/L MgCl₂, 1 mmol/L ethylenediamine tetraacetate, 0.1% BSA, and 0.1 mg/mL bacitracin. Incubations were carried out for 1.5 h at 22 C in a 0.25-mL volume supplemented with 40,000 cpm [125I]bTSH (54 μCi/μg; Brahms Diagnostika). Membranes and bound ligand were separated from unbound ligand by centrifugation (10,000 × g, 10 min) through a silicon oil layer according to the method of McArdle (21). Nonspecific binding was defined as binding in the presence of 100 nmol/L bTSH. The protein content of samples was determined by the method of Bradford (22). Binding data were analyzed by a nonlinear least squares curve-fitting procedure using the computer program Ligand (23).

Immunofluorescence microscopy and enzyme-linked immunosorbent assay (ELISA)

Immunofluorescence microscopy was performed as described previously (24). A 1:4 dilution of tissue culture supernatant from the hybridoma clone 2C11 directed against the extracellular domain of the human TSHR (25) was used to detect receptor protein. For ELISA studies, COS-7 cells were transfected with various TSHR constructs as described above. Three days later, cells were washed twice with PBS and treated with 120 μL lysis buffer [10 mmol/L Tris-HCl (pH 7.4), 150 mmol/L NaCl, 1 mmol/L diithiothreitol, 1 mmol/L ethylenediamine tetraacetate, 1% deoxycholate, 1% Nonidet P-40, 0.2 mmol/L phenylmethylsulfonyl fluoride, and 10 μg/mL aprotinin]. After vigorous vortexing, followed by removal of cell debris by centrifugation, supernatants (100 μL/well) containing solubilized receptor protein were used to coat microtiter plates. After incubation for 16 h at 4 C, plates were blocked with 10% FCS in PBS. Then, 100 μL/well of a 1:4 dilution of tissue culture supernatant from the hybridoma clone 2C11 directed against the extracellular domain of the human TSHR (25) was used to detect receptor protein.
the addition of 1 mol/L H2SO4 containing 0.05 mol/L Na2SO3, and color development was measured bichromatically at 492 and 620 nm using an ELISA reader (Titertek Multiskan MCC/340, Labsystems, Finland).

Results

Evaluation of clinical endocrine parameters

At birth, the patient (daughter 1) presented with markedly elevated TSH levels concomitant with low peripheral thyroid hormone levels and a reduced thyroid volume as determined by ultrasonography. This constellation of clinical findings warranted the diagnosis of congenital hypothyroidism. The results of thyroid function tests and ultrasonographic examinations of the patient at the time of initial diagnosis and during the follow-up period are summarized in Table 1. At the age of 6 months, the daily T4 dose was increased to 62.5 μg to counteract elevated TSH levels and undetectable FT4 serum concentrations measured during low dose therapy and was maintained until the age of 2 yr. Clinical data obtained during a 4-week discontinuation of T4 replacement therapy (Table 1) confirmed the initial diagnosis, and L-T4 therapy was reinstated.

In the mother 2 yr before her first pregnancy, a moderately elevated serum TSH concentration (5.5 mU/L; normal, <4 mU/L) and a borderline FT4 level of 10.5 pmol/L (normal, 11–25 pmol/L) had been reported by her physician. Ultrasonographic examinations at that time indicated a rather small thyroid volume of 5 mL. Tests for antithyroid peroxidase, antithyroglobulin, and TSH binding inhibitory antibodies yielded negative results, and she had been treated with a daily L-T4 dose of 75 μg since then. When reexamined by us at the age of 29 yr, discontinuation of L-T4 treatment for 6 weeks was accompanied by TSH levels in the normal range (Table 1). The father, who was of Romanian origin, and the presently 1-yr-old second daughter were euthyroid.

Genomic analysis and identification of mutations in the TSHR gene

Applying SSCP analysis, genomic DNA of the patient (daughter 1; Fig. 2A) was screened for mutations in exons 1–10 of the TSHR gene. PCR products representing a 376-bp segment of exon 10 of the TSHR gene showed marked differences in electrophoretic mobility compared with amplificates from healthy controls (Fig. 2B). Further studies of maternal and paternal TSHR genes identified the parents as heterozygote carriers of 2 different abnormalities (Fig. 2B). Genomic DNA amplified from the younger daughter (daughter 2; Fig. 2A) displayed an electrophoretic mobility profile identical to that of the father, whereas DNA from the patient appeared to combine properties of both parents. Subsequently, a 376-bp fragment of exon 10 of the TSHR gene was sequenced. Different alterations were detected in each of the 2 alleles, confirming the initial suspicion of compound heterozygocity (Fig. 3). In 1 allele, 18 bp (nucleotide positions 1217–1234) were deleted, and 4 novel bp were inserted instead (Fig. 3). These alterations resulted in a frame shift and the appearance of coding sequence for 14 novel amino acids, followed by a premature stop codon after amino acid position 419 (Fig. 1). The rearrangement of exon 10 within 1 allele created a novel unique BsaAI restriction site in the 376-bp PCR fragment that could now be used to trace this mutant to the mother (Fig. 2C). Subsequent sequencing of the paternal allele revealed a T to G transversion at nucleotide position 1170, resulting in the exchange of a highly conserved C at position 390 for a W residue (Figs. 1 and 3). Sequencing further confirmed the heterozygous state of each parent and that of daughter 2 by the presence of 1 normal allele.

To exclude the possibility of additional TSHR mutations that were missed by SSCP analysis and could potentially contribute to the clinical picture, exons 1–10 of the TSHR gene were amplified (13) and sequenced. No additional mutations were found.

Functional characterization of mutant TSHRs

To characterize the functional activities of mutant TSHRs, the wild-type TSHR cDNA was modified by site-directed mutagenesis. In transiently transfected COS-7 cells, stimulation of the wild-type receptor and of the paternal mutant TSHR-C390W with saturating concentrations of TSH resulted in a robust increase in agonist-induced cAMP accumulation (Table 2). However, dose-response curves for TSH were shifted toward higher agonist concentrations in the case of TSHR-C390W compared to the wild-type re-
ceptor (Fig. 4A), whereas the maximum number of binding sites was comparable for both receptors (Table 2). The approximately 20-fold greater potency of TSH acting at the wild-type compared to the mutant (TSHR-C390W) receptor was the functional correlate of binding characteristics that were monitored in parallel (Fig. 4B). The concentration of TSH required to displace 50% of specifically bound \(^{125}\text{I}\)bTSH was 16-fold higher for the paternal mutant than for the wild-type receptor (Fig. 4B and Table 2), thus reflecting a significantly reduced affinity of TSH to the mutant receptor. Expression of the truncated TSHR derived from the altered maternal allele did not yield agonist-induced cAMP accumulation (Fig. 4A) or detectable membranous binding sites for \(^{125}\text{I}\)bTSH (Table 2).

**TABLE 2.** Functional properties of wild-type and mutant TSH receptors transiently expressed in COS-7 and permanently expressed in CHO-K1 cells

<table>
<thead>
<tr>
<th>Transfected cells</th>
<th>Binding</th>
<th>cAMP determination</th>
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<tr>
<td></td>
<td>(B_{\text{max}}) (fmol/mg protein)</td>
<td>(K_i) for (b)TSH (nmol/L)</td>
</tr>
<tr>
<td>COS-7 (transient)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>wtTSH-R</td>
<td>12.0 ± 2.0</td>
<td>0.19 ± 0.02</td>
</tr>
<tr>
<td>TSHR-C390W</td>
<td>17.7 ± 6.7</td>
<td>2.98 ± 0.77</td>
</tr>
<tr>
<td>TSHR-419trunc</td>
<td>—(^a)</td>
<td>—(^b)</td>
</tr>
<tr>
<td>CHO-K1 (permanent)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>wtTSHR</td>
<td>14.5 ± 4.5</td>
<td>0.06 ± 0.01</td>
</tr>
<tr>
<td>TSHR-C390W</td>
<td>100 ± 50</td>
<td>4.71 ± 2.2</td>
</tr>
<tr>
<td>TSHR-419trunc</td>
<td>—(^a)</td>
<td>—(^b)</td>
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Wild-type and mutant TSH receptors were transiently expressed in COS-7 cells and permanently expressed in CHO-K1 cells. Functional studies were performed as indicated in Subjects and Methods. Maximal increases in cAMP levels were determined in the presence of 1 \(\mu\)mol/L bTSH. Data are the mean ± SEM of three independent experiments, each performed in triplicate.

\(^a\) No specific membrane binding sites.

\(^b\) No significant increase in cAMP above basal levels.
transfection of the wild-type TSHR with each of the mutant receptors to simulate the heterozygous state of the parents resulted in reduced efficacy, as expected because of diminished gene dosage, yet unaltered potency of TSH to stimulate cAMP accumulation (data not shown).

Apart from adenylyl cyclase, phospholipase C has also been shown to be activated by TSH in the human thyroid (2) as well as in CHO-K1 cells permanently expressing the human TSHR (26). Transient expression of the mutant receptors in COS cells, however, did not result in agonist-dependent inositol phosphate formation upon stimulation with saturating concentrations of TSH (1 μmol/L).

In transient transfection experiments, only a small percentage of cells take up expression plasmids and subsequently show unphysiologically high receptor densities. As signal transduction characteristics vary considerably depending on the amount of receptors expressed per cell (27), CHO-K1 cell lines were created that permanently expressed TSHR levels comparable to those observed in thyrocytes (28).

Several G418-resistant CHO cell lines were screened for receptor expression by radioligand binding and functional techniques, and clonal lines were developed. None of the CHO cell clones that had previously been shown to express TSHR-419trunc messenger ribonucleic acid with the help of reverse transcription PCR (data not shown) displayed specific membraneous [125I]bTSH-binding sites or TSH-stimulable adenylyl cyclase activity (Fig. 4C). In accordance with data obtained in COS-7 cells, the concentration of TSH required to half-maximally increase cAMP accumulation was approximately 20-fold higher in cells expressing the wild-type receptor compared to those expressing the TSHR-C390W mutant (Fig. 4C). It is noteworthy, however, that to be able to detect agonist-induced cAMP accumulation in stably transfected CHO cells at all, TSHR-C390W clones had to be chosen that display 5- to 7-fold higher receptor densities than wild-type clones (Table 2). Even under those conditions, the efficacy of TSH acting at the mutant is considerably lower than that of the wild-type receptor (Fig. 4C). In binding studies performed in parallel, the reduced potency and efficacy of TSH interacting with the paternal mutant receptor were reflected by a drastically reduced affinity of TSHR-C390W to [125I]bTSH (Fig. 4D).

Cellular localization of wild-type and truncated TSHR

As altered or absent functional characteristics of mutated TSHRs could potentially be related to aberrant cellular protein expression, we set out to study the expression and subcellular localization of TSHR by confocal fluorescence microscopy using a monoclonal antibody directed against the extracellular domain of the human TSHR (25). Nonperme-
abilized COS-7 cells transfected with a cDNA coding for the wild-type TSHR and for TSHR-C390W showed an intense staining of the plasma membrane (Fig. 5, A and C), whereas cells transfected with TSHR-419trunc cDNA completely lacked any cell surface staining (Fig. 5E). Studies with permeabilized cells, however, revealed that considerable amounts of wild-type and mutant TSHRs were retained inside the cell (Fig. 5, B, D, and F). It should not go unnoticed, however, that a high percentage of cells expressing the truncated receptor protein contained large immunoreactive conglomerates, preferably located in the vicinity of the nucleus (Fig. 5F).

To directly compare the amount of receptor protein synthesized after transfection of wild-type or mutated TSHR constructs, we devised an ELISA system taking advantage of a monoclonal antibody that recognizes an epitope in the N-terminal region of the human TSHR (25). After transient transfection of COS-7 cells, all three receptor proteins (wild-type, TSHR-C390W, and TSHR-419trunc) were expressed at similar levels (optical density at 492 nm = 0.7–1.1).

**Discussion**

The importance of TSH for normal thyroid development is substantiated by the hyt/hyt mouse model characterized by a loss of function mutation in the TSHR resulting in severe hypothyroidism.
hypothyroidism with thyroid hypoplasia (6, 7). A similar phenotype, consisting of thyroid hypoplasia and severe congenital hypothyroidism, was described in TSH-deficient mice after targeted disruption of the α-subunit gene of glycoprotein hormones (29). The demonstration of the first expression of the TSHR gene on day 14 of mouse development, a time when the thyroid anlage has just arrived in the position of the anterior neck and differentiation of thyroid follicles starts (5), suggests that TSH function might not be important for the early events of thyroid development, but may regulate the subsequent growth and differentiation of the gland. Thus, the clinical consequence of loss of function mutations may cover a range from hyperthyrotropinemia to hypoplasia with hypothyroidism, but not to athyrosis or ectopy. Assuming that TSH plays a critical role in promoting growth as well as differentiation of the human thyroid gland, the TSHR can be considered a candidate gene, possibly altered in congenital hypothyroidism due to hypoplasia of the gland (30). Therefore, we screened for mutations in the TSHR gene in patients with congenital hypothyroidism and thyroid hypoplasia. Two novel TSHR mutants were identified in a girl suffering from congenital hypothyroidism. She is a compound heterozygote, having inherited distinct defective alleles from either parent.

Functional characterization of the mutant paternal TSHR harboring a missense mutation at nucleotide position 1170 revealed a loss of affinity and a subsequent loss of potency of TSH acting at the mutant TSHR-C390W. The human TSHR contains 11 cysteine residues in the N-terminal extracellular domain that are assumed to be involved in disulfide bonding to preserve the overall three-dimensional structure of the ligand-binding domain and to attach the extracellular α-subunit to the membrane-spanning β-subunit after cleavage of the receptor protein in the cell membrane (31). C-390 is strictly conserved within the family of glycoprotein hormone receptors (3). It was speculated that C-301 and C-390 are linked by a disulfide bond to preserve a protein conformation required for high affinity hormone interaction and for binding of blocking anti-TSHR antibodies (32). Thus, it was not surprising that the disruption of a putative disulfide bridge and the introduction of a bulky hydrophobic indol ring into a hydrophilic environment, as realized in the mutant TSHR-C390W, interfered with hormone binding and signal transduction of the mutant receptor. In consonance with our findings, mutation of C-390 to ser decreased the affinity of the receptor for TSH and the potency of the hormone in terms of cAMP formation approximately 25- to 30-fold (32).

It is noteworthy, however, that the phenotype of the maternal mutation at the cellular level was more prominent in CHO cells permanently expressing TSHRs than in transiently transfected COS-7 cells. In the case of mutant V2 vasopressin receptors, it has been shown that functional data obtained in COS-7 cells may differ dramatically from those derived from stable cell lines (33). Although only a small fraction of COS cells incorporate TSHR cDNA during transient transfection procedures, the few cells that become transfected will substantially overexpress receptor protein. Under such circumstances it has frequently been found that an increase in receptor density is associated with a decrease in the EC₅₀ value for the agonist, and artifactual G protein coupling patterns may occur (summarized in Ref. 27). Thus, after transient transfection of COS cells, caution has to be exercised when interpreting signal transduction processes in physiological terms. CHO cell lines chosen for our studies represent homogeneous cell populations expressing wild-type TSHRs at physiological densities (28). In contrast to studies with the wild-type receptor, TSH-induced cAMP accumulation via the mutant TSHR-C390W was hardly detectable if expressed at comparably low densities (10–50 fmol/mg protein). Therefore, we had to resort to a stable cell line homogeneously expressing higher densities of mutant receptors to functionally characterize TSHR-C390W. In summary, one may surmise that in thyroid follicular cells, signal transduction involving TSHR-C390W is severely impaired regardless of elevated serum TSH concentrations.

The mutant maternal allele described in the present study codes for a truncated receptor protein that comprises most of the N-terminal hormone-binding domain (TSHR-419trunc). In mammalian cells it has been difficult to produce conformationally intact TSHR ectodomain that still had the ability to bind hormone and were secreted from the cell (34, 35). Truncated LH/CG receptors, however, displayed high affinity hormone binding, but were also reported by some researchers to be trapped intracellularly (36, 37), although other investigators described the secretion of truncated LH/CG receptors (38, 39). Immunofluorescence studies revealed that TSHR-419trunc cDNA was transcribed and translated. Most noticeably, the truncated protein was retained intracellularly and was not inserted into the plasma membrane. As TSHR-419trunc cDNA encodes the extracellular TSHR domain nearly in its entirety, including the signal sequence for translocation through endoplasmic reticulum (ER) membranes, it appears reasonable to assume that the reticular fluorescence pattern observed resulted from synthesis of the truncated protein at the rough ER. Failure of an aberrant protein to fold correctly may lead to retention in the ER (40), resulting in the formation of large immunopositive intracellular aggregates, as observed by us in transfected COS-7 cells.

Hypothyroidism of the patient, as assessed by thyroid function tests, was not as severe as that in patients suffering from athyrosis or profound defects of thyroid hormone biosynthesis. Serum T₄ concentrations at the time of clinical diagnosis and after discontinuation of replacement therapy were within the range determined in patients with thyroid ectopy (32–130 nmol/L) or thyroid dysmorphogenesis (20–154 nmol/L) (41). During hormone replacement therapy, close monitoring and a steady increase in the dose of l-T₄ was necessary, because a decrease in the T₄ dose per kg BW was accompanied by a pronounced increase in serum TSH levels and a decrease in T₃ and free T₄ levels, probably reflecting the limited biosynthetic capacity of the hypoplastic gland. This observation is at variance with other cases of mild hypothyroidism, for instance due to thyroid ectopy, which can be treated with lower daily l-T₄ doses for the first 3 yr of life without evidence of decreasing T₄ levels (42). In summary, the diagnosis of persistent congenital hypothyroidism is substantiated by clinical and biochemical findings. These clinical data are in good agreement with results obtained in in vitro expression experiments that emphasize a severely impaired
signal transduction via mutant TSHRs. The low residual T4 secretion observed in our patient may result from the residual signal transduction capability of the paternal TSHR-C390W and may reflect basal T4 secretion as well.

The father and one younger sister who bear the TSHR-C390W mutation were euthyroid. The mother carrying the TSHR-419trunc allele, however, was reported to have undergone a period of mild hypothyroidism in the past. Upon reexamination by us, the latter diagnosis could not be confirmed. However, we cannot rule out that in certain situations, such as puberty, pregnancy, or iodine deficiency, wild-type/TSHR-419trunc heterozygocity may develop the clinical correlate of hyperthyrotropinemia or even mild hypothyroidism. These observations notwithstanding, genetic and clinical studies suggest an autosomal recessive mode of inheritance of congenital hypothyroidism in the affected family.

The phenotype of this patient with congenital hypothyroidism and thyroid hypoplasia differs from the clinical picture of three siblings identified to be compound heterozygote for two different TSHR mutations in the extracellular domain (P162A/I167N), who were characterized to have normal thyroid function with elevated TSH levels and normal thyroid volume (9). At the time of submission of our manuscript, de Roux et al. (43) reported on four new cases of hyperthyrotropinemia due to mutant TSHRs. Because of these different phenotypes we would like to put forward the hypothesis that loss of function mutations in the TSHR are unlikely to provide a molecular explanation for the majority of patients suffering from congenital hypothyroidism because most of them are characterized by thyroid ectopy or athyrosis. Moreover, the congenital hypothyroidism because most of them are characterized by thyroid ectopy or athyrosis. Furthermore, we cannot rule out that in certain situations, such as puberty, pregnancy, or iodine deficiency, wild-type/TSHR-C390W heterozygocity may develop the clinical correlate of hyperthyrotropinemia or even mild hypothyroidism. These observations notwithstanding, genetic and clinical studies suggest an autosomal recessive mode of inheritance of congenital hypothyroidism in the affected family.

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In the present study we demonstrate for the first time that germ-line loss of function mutations in the TSHR gene may result in persistent congenital hypothyroidism due to hypoplasia of the thyroid gland. However, loss of function mutations in the TSHR are unlikely to provide a molecular explanation for the majority of patients suffering from congenital hypothyroidism because most of them are characterized by thyroid ectopy or athyrosis. Moreover, the autosomal recessive inheritance demonstrated for TSHR loss of function mutations is incompatible with the hallmark of sporadic occurrence in almost all cases with congenital hypothyroidism, implying other genetic mechanisms or environmental factors as the cause of thyroid dysgenesis.

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