Long-Term Outcome of Loss-of-Function Mutations in Thyrotropin Receptor Gene

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Background: Loss-of-function mutations in the thyrotropin receptor (*TSHR*) gene lead to resistance to TSH (RTSH) presenting with either congenital hypothyroidism (CH) or subclinical hypothyroidism (SCH). Despite several reports of patients with *TSHR* mutations, data on the long-term outcome of this condition are limited, and no consensus exists on the need for hormone replacement therapy. The aim of the present study was to assess the long-term outcome in children and adolescents with RTSH due to *TSHR* mutations.

Methods: The *TSHR* gene was sequenced in 94 subjects (aged 3 days–21 years) with either nonautoimmune SCH or CH with RTSH.

Results: Twenty-seven subjects (29%) carried mutations in *TSHR*. Fifteen infants were identified by neonatal screening, and the other 79 patients were detected in the process of testing for various other conditions or because of family occurrence of thyroid test abnormalities. Six different mutations were identified: c.484C>G (p.P162A), c.202C>T (p.P68S), c.790C>T (p.P264S), c.269A>C (p.Q90P), c.1957C>G (p.L653V), and c.1347C>T (p.R450C). Twelve subjects were homozygous, three were compound heterozygous, and 12 were heterozygous. Mean serum TSH levels at diagnosis and at last visit were significantly higher in patients with *TSHR* mutations than in those without mutations (29.04 vs. 14.15, $p=0.002$; 31.73 vs. 6.19, $p<0.0001$, respectively). Homozygous patients had a more severe phenotype (TSH 53.6 vs. 9.24, $p<0.0001$). Mean serum free thyroxine (fT4) levels at the last visit were significantly lower than at the first visit in the homozygous individuals ($p=0.05$) for a follow-up period of as long as 11 years. Heterozygous subjects had only mild hyperthyrotropinemia with stable TSH levels. However, homozygous subjects showed a trend toward increased TSH and decreased fT4 with time.

Conclusion: SCH in heterozygotes with *TSHR* mutations is a stable compensated condition with an appropriately adjusted set point for pituitary–thyroid feedback that does not require replacement therapy. However, homozygous subjects, with incompletely compensated SCH, show reduced fT4 levels over time and may require levothyroxine treatment. Replacement therapy should be considered on an individual basis, and long-term follow up is recommended.

Introduction

RESISTANCE TO THYROTROPIN (RTSH) is characterized by elevated serum TSH levels, normal or low triiodothyronine (T3) and/or thyroxine (T4) concentrations, and a eutopic, normal, or hypoplastic thyroid gland. Loss-of-function mutations in the TSH receptor (*TSHR*) gene lead to RTSH characterized by either congenital hypothyroidism (CH) or subclinical hypothyroidism (SCH) (1–6).

The definition of SCH is a serum TSH concentration above the upper limit of the reference range, with fT4 and fT3 levels within the reference range. It suggests a compensated state of primary thyroid failure requiring increased levels of TSH to maintain normal levels of thyroid hormones (7).

To date, about 60 different loss-of-function mutations in the *TSHR* have been described (4–6). The degree of hyposensitivity to TSH depends on the type and location of the *TSHR* mutation and whether the patient is homozygous or

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heterozygous. More severe loss of *TSHR* function manifests as CH, whereas mild or heterozygous mutations present as SCH in childhood or adulthood.

The prevalence of inactivating *TSHR* mutations differs among populations, based on the selected clinical criteria, and on whether the subjects present with CH or SCH. A prevalence of 4.3% biallelic *TSHR* mutations has been reported among Japanese infants with CH (8), whereas a high prevalence of 29% has been found among Italian children with nonautoimmune SCH (9).

Despite several reports of subjects with *TSHR* mutations, limited data are available on the long-term outcome of this condition, and no consensus exists on the need for supplemental thyroid hormone therapy. In a cross-sectional analysis of an extended family with 33 members having *TSHR* mutations, it has previously been shown that TSH levels are stable with age, suggesting permanently compensated RTSH with an appropriately adjusted set point for pituitary–thyroid feedback (10).

In the current study, the long-term outcome of 94 patients presenting with RTSH characteristics, with or without *TSHR* mutations, for up to 20 years was assessed. This large sampling and long-term observation enabled the delineation of the clinical course of SCH in children.

Subjects and Methods

Patients

Ninety-four subjects were included in the study (54 males) with a mean age at presentation of 52 months (range 3 days–21 years). Selection of subjects for *TSHR* sequencing was based on the following criteria: (a) subjects with TSH levels above the upper limit of the reference range (>4.2 mIU/L) in at least two separate determinations with normal thyroid hormone levels; (b) infants identified by national neonatal screening for hypothyroidism in whom persistent elevation of TSH with normal or slightly below normal range fT4 values were observed after replacement treatment was withdrawn (replacement therapy is withdrawn at the age of two to three years, as per the routine management of infants with CH); (c) ectopic thyroid gland of normal size or hypoplastic on ^{99}Tc pertechnetate (^{99}Tc) scan, or lack of a thyroid gland on ^{99}Tc scan but a normal or hypoplastic gland by ultrasonography (ultrasonographic imaging was performed when the ^{99}Tc scan showed lack of a gland with measured serum thyroglobulin); and (d) siblings of subjects with SCH or identified as having *TSHR* mutations. Subjects were excluded under the following criteria: (a) positive autoantibodies to thyroid peroxidase (TPO) and thyroglobulin (TG); and (b) known genetic conditions or chromosomal abnormalities. All patients were followed at 3- to 12-month intervals for as many as 20 years, and thyroid function tests were performed at three- to six-month intervals. Thyroid morphology was evaluated by scintigraphy in all patients with CH. All of the clinical parameters were collected retrospectively from the medical records, and results of biochemical tests were collected from the health-insurance companies' computerized systems. All data presented in this study were obtained from patients while off levothyroxine (LT4) therapy.

The phenotypes of some of these subjects have been previously described (10,11), but in the present study, the aim

was to assess the long-term outcome in these individuals along with additional subjects with RTSH characteristics.

Written informed consent was obtained from all participants. This study was approved by the Ethics Committee of Ha'Emek Medical Center, the Genetics Committee of the Israeli Ministry of Health, and the Institutional Review Board of the University of Chicago.

Hormonal analysis

TSH, fT4, FT3, and TG and TPO antibodies were measured by direct automated chemiluminescent immunoradiometric assays using ADVIA Centaur (Bayer Corporation, Tarrytown, NY). Serum TG levels were measured by a direct automated chemiluminescent immunoradiometric assay using Immulite 2000 (Siemens, Llanberis, Gwynedd, United Kingdom).

National neonatal screening

The Israeli National Newborn Screening Program for CH was implemented in 1987. Blood samples are collected by heel puncture 48–72 h after birth. Between 1987 and 2006, the Israeli national newborn screening laboratory performed the tests using DPC (Los Angeles, CA) radioimmunoassay. Since 2006, measurements have been made by PerkinElmer B065-112 AutoDELFIA neonatal total thyroxine (TT4) and B032-312 AutoDELFIA neonatal TSH, both using time-resolved fluoroimmunoassays. The Israeli CH screening program is based on a TT4 test followed by confirmatory TSH test. Therefore, when the level of TT4 is below the daily 10th percentile, TSH is measured. TSH values >20 mIU/L are considered indicative of primary CH. Neonates with abnormal screening results are referred to medical centers. Negative screening results were retrieved retrospectively from the national computerized database.

Molecular analysis

Genomic DNA was isolated from peripheral leucocytes by standard methods. All coding regions of the *TSHR* gene were amplified (sequences available on request). Sequencing reactions for both DNA strands were performed with the Big-Dye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems, Foster City, CA) and analyzed with an automatic sequencer (Applied Biosystems). The *TSHR* coding regions of the 94 subjects enrolled in the study were sequenced.

Statistical analysis

Statistical analyses were performed with SAS v9.2 statistical software package (Cary, NC). Groups were compared using the Student's *t*-test. When the Student's *t*-test assumptions were not valid for continuous variables, the Wilcoxon two-sample test was used. The chi-square test (or Fisher's exact test) was used for categorical variables. Significance was set at $p < 0.05$. Scatter plots were generated for TSH and fT4 levels versus age. Loess smoothing was used to express trends with age. The Wilcoxon sign rank test was used to determine the difference in TSH and fT4 levels between the first and last measurements in patients with *TSHR* mutations.

Results

Ninety-four subjects were included in the study. Of these, 88 were of Arab-Muslim origin with a high incidence of consanguinity, and six were of Jewish origin. Seventy-nine subjects had SCH, and 15 were identified by national neonatal screening as having CH (Table 1). *TSHR* mutations were identified in 27 subjects (29%). Mean serum TSH levels were significantly higher both at diagnosis ($p=0.002$) and at the last visit ($p<0.0001$) in patients with *TSHR* mutations compared to those without mutations (Table 1). Mean serum fT4 concentrations did not differ at presentation but were slightly lower at the last visit in subjects with *TSHR* mutations ($p=0.0195$; Table 1). The difference in TSH concentration (Δ TSH) between the first and last visit was significantly greater in subjects with *TSHR* mutations ($p<0.002$), but the Δ fT4 did not differ (Table 1). The evolution of TSH and fT4 values over the years, for both groups, is shown in Figure 1.

Six different mutations were identified: c.484C>G (p.P162A), c.202C>T (p.P68S), c.790C>T (p.P264S), c.269A>C (p.Q90P), c.1957C>G (p.L653V), and c.1347C>T (p.R450C). Five of these have been previously described (12–14), and one was novel (Table 2). Twelve subjects were homozygous, three were compound heterozygous, and 12 were heterozygous. Twelve single-nucleotide polymorphisms (SNPs) were identified, consisting of 10 known and two unknown variants (Supplementary Table S1; Supplementary Data are available online at www.liebertpub.com/thy). All but one of the subjects harboring *TSHR* mutations were of Arab-Muslim origin, belonging to four extended families. Age at

first presentation ranged from 3 days to 21 years, with five infants identified by neonatal screening. The others were detected in the process of testing for various other conditions or because of family occurrence of thyroid test abnormalities. In the five infants who were detected by neonatal screening (four homozygous and one heterozygous), repeated thyroid tests in the authors' laboratory, prior to initiation of LT4 therapy, revealed normal fT4 levels in all except case 9, and four were treated with LT4. Thyroid tests at two years of age, after LT4 withdrawal, showed SCH, and only case 9 had persistent hypothyroidism (Table 3).

Since the Israeli national screening is based on TT4 measurements, test results from the 22 subjects who were not detected by the screening were retrieved retrospectively from the national database. In these cases with normal TT4 levels, TSH was not tested. Mean screening TSH and TT4 levels did not differ between subjects with or without *TSHR* mutations. Thyroid glands were of normal size or hypoplastic but in a normal location in the subjects with *TSHR* mutations, as demonstrated by ^{99}TC scan or by ultrasonography. Only one patient (case 18) had an enlarged lobe (Table 3).

All homozygous and 30% of the heterozygous subjects were consanguineous. Homozygotes had a more severe phenotype manifested by higher TSH levels at diagnosis and at last visit (mean TSH 53.6 vs. 9.24, $p<0.0001$; 62.07 vs. 9.79, $p=0.0005$, respectively). Despite the significantly higher serum TSH concentrations, mean serum fT4 levels did not differ between homozygotes and heterozygotes (13.96 vs. 16.27 at diagnosis, $p=0.175$; 12.53 vs. 14.35 at last visit, $p=0.1006$, respectively). Compound heterozygous subjects

TABLE 1. CHARACTERISTICS OF ALL PATIENTS (94 SUBJECTS) AND COMPARISONS BETWEEN SUBJECTS WITH AND WITHOUT *TSHR* GENE MUTATIONS

Parameter	Total (mean $\pm$ SDS) [median] (range)	<i>TSHR</i> mutation	Without <i>TSHR</i> mutations	p-Value	Normal range
Sex (female/male)	40(42%)/54(58%)	13(48%)/14(52%)	27(40%)/40(60%)	0.4861	
Arab/Jewish	88 (94%)/6 (6%)	26 (96%)/1(4%)	61 (91%)/6 (9%)	0.1772	
Consanguinity (%)	35 (39%)	16 (64%)	19 (29%)	0.0024*	
Age at presentation (years)	4.4 $\pm$ 4.9 [2.2] (0.01–21.8)	5.1 $\pm$ 5.4 [3.65] (0.01–21.8)	4.2 $\pm$ 4.7 [2.01] (0.01–16.7)	0.4906	
SCH detected later in life	79 (84%)	22 (81%)	57 (85%)	0.1727	
Neonatal screening	15 (16%)	5 (19%)	10 (15%)		
TSH (mIU/L) at presentation	18.47 $\pm$ 23.72 [9.0] (4.22–134)	29.04 $\pm$ 27.09 [13.5] (4.22–91)	14.15 $\pm$ 20.92 [8.33] (5.12–134)	0.0020*	0.27–4.2
fT4 (pmol/L) at presentation	15.78 $\pm$ 4.89 [15.1] (0.52–46.49)	15.11 $\pm$ 3.11 [14.87] (9.5–24.81)	15.29 $\pm$ 3.14 [15.4] (0.52–46.49)	0.2682	12.0–22.0
TSH (mIU/L) at last visit	13.57 $\pm$ 23.17 [5.45] (2.32–138.51)	31.73 $\pm$ 36.43 [13.97] (3.69–138.51)	6.19 $\pm$ 6.37 [4.83] (2.32–47.83)	<0.0001*	0.27–4.2
fT4 (pmol/L) at last visit	14.51 $\pm$ 2.58 [14.45] (6.8–23.2)	13.45 $\pm$ 2.43 [13.52] (9.0–17.09)	14.96 $\pm$ 2.52 [14.82] (6.8–23.2)	0.0195*	12.0–22.0
Δ TSH	–0.24 $\pm$ 17.71 [–1.63] (–65.93–97.26)	5.8 $\pm$ 26.95 [2.17] (–61.44–97.26)	–2.62 $\pm$ 11.84 [–2.43] (–65.93–41.62)	0.0016*	
Δ fT4	–1.55 $\pm$ 5.51 [–0.46] (–32.5–8.53)	–1.54 $\pm$ 3.32 [–1.6] (–12.35–2.89)	–1.55 $\pm$ 6.33 [–0.3] (–32.5–8.53)	0.3945	
LT4 therapy (no. patients)	17 (18%)	9 (34%)	8 (11.76%)	0.0347*	
TSH (mIU/L) screening	37.66 $\pm$ 52.32 [21.5] (1.9–250)	38.06 $\pm$ 20.8 [40.0] (12.6–67)	37.51 $\pm$ 60.93 [18.95] (1.9–250)	NS	> 24
TT4 (μ g/dL) screening	10.08 $\pm$ 3.48 [9.8] (2.8–18.92)	9.32 $\pm$ 4.05 [9.6] (2.8–17.47)	10.43 $\pm$ 3.21 [9.85] (4.7–18.92)	NS	> 10

* $p < 0.05$.

Δ TSH, TSH at last visit – TSH at presentation; Δ fT4, fT4 at last visit – fT4 at presentation.

TSHR, thyrotropin receptor; SCH, subclinical hypothyroidism; fT4, free thyroxine; LT4, levothyroxine; NS, not significant.

FIG. 1. Thyrotropin (TSH) and free thyroxine (FT4) concentrations as a function of age in subjects without (left panel) and with (right panel) thyrotropin receptor (*TSHR*) gene mutations. Dashed lines represent age-related reference ranges (15).

had one severe mutation and one very mild one (L653V/P68S) presenting with a moderate phenotype, reflecting the weak functional effect of the P68S mutation (9,11). They were therefore not included in the comparison between the homozygotes and heterozygotes. Heterozygous subjects had

TABLE 2. SUMMARY OF IDENTIFIED *TSHR* GENE MUTATIONS

DNA	Protein	Exon	No. of alleles	Reference
c.202C > T	p.P68S	2	5	10
c.269A > C	p.Q90P	3	6	11
c.484C > G	p.P162A	6	6	3
c.790C > T	p.P264S	9	6	11
c.1957C > G	p.L653V	10	23	10
c.1348C > T	p.R450C	10	2	Novel

mild hyperthyrotropinemia with TSH levels that remained stable over time (Fig. 2). In homozygous subjects, a slight decrease in FT4 with the passage of time was accompanied by a slight increase in TSH (Fig. 2). In some of these individuals, FT4 levels declined below the lower normal limit for age. The mean serum FT4 level in homozygous subjects was significantly lower at the last visit compared to the first visit ($p=0.05$), but did not differ in the heterozygous subjects in a follow-up period of 11 years. All subjects tested negative for TPO and TG antibodies at presentation and in repeated testing until the last visit in all but one subject (case 15) who tested positive for TG antibodies at her last visit. Three patients (patients 15, 17, and 18; Table 3) carrying homozygous *TSHR* mutations, in whom an elevated TSH persisted for as long as nine years, underwent a brain magnetic resonance imaging scan that demonstrated normal-sized pituitary

TABLE 3. CHARACTERISTICS OF SUBJECTS WITH *TSHR* GENE MUTATIONS

Case	Family	M/F	Neonatal screening			At presentation				Last visit				Imaging	
			TSH (mIU/L)	TT4 (μg/dL)	Age (months)	Cause for preforming thyroid tests	TSH (mIU/L)	fT4 (pmol/L)	Age (years)	TSH (mIU/L)	fT4 (pmol/L)	LT4 therapy	Genotype	⁹⁹ TC	US
1 ^a	1	F	40	4.7	0.5	Screening	30.75	13.77	6.66	19.79 ^b	16.35 ^b	0.46m	L653V/L653V	Normal	ND
2 ^c	1	M	ND	12.9	151	Familial	61.25	9.52	13	39	11.45	—	L653V/L653V	Normal	3 mm echogenic area
3	1	M	43	5.7	1.0	Screening	72.45	16.39	6.0	14.63 ^b	16.71 ^b	1m	L653V/L653V	Low trapping	ND
4	1	M	NA	NA	0.5	TF	10.32	20.89	1.6	14.19	15.45	—	L653V/W	ND	ND
5	2	F	NA	13.5	43.2	Familial	59.97	17.54	3.8	49.71	17.09	—	P162A/P162A	ND	ND
6	2	F	NA	10.4	74.4	Familial	32.9	14.74	6.3	42.76	15.71	—	P162A/P162A	ND	ND
7	2	F	21.5	5.4	126	TF	17.53	14.4	12.25	9.29	16.29	11.8y	P162A/P162A	ND	ND
8	3	F	ND	11.8	44.4	TF	5.42	13.93	13.11	7.14	16.82	—	P68S/W	ND	Normal
9	4	M	67	2.8	0.7	Screening	68.3	5.34	7.3	6.86 ^b	12.98 ^b	0.7m	Q90P + P264S/Q90P + P264S	Low trapping	ND
10	4	F	NA	NA	18	TF	13.5	18.58	5.4	13.79 ^b	16.84 ^b	18m	Q90P + P264S/W	Normal	Normal
11	4	M	12.6	13.0	0.3	Familial	11.84	15.5	6	14.14	13.9	—	Q90P + P264S/W	ND	ND
12	4	M	ND	17.47	0.1	Familial	16.3	24.8	0.33	6.26	15.65	—	Q90P + P264S/W	ND	ND
13	4	F	ND	10.9	1	Neonatal jaundice	9.0	16.6	11.3	13.6	12.85	1m	Q90P + P264S/W	Normal	Normal
14	5	M	NA	NA	47.3	Short stature	10.54	15.1	9.6	8.1	13.9	—	L653V/P68S	Normal	Normal
15 ^{d,e}	5	M	NA	NA	252	Familial	84	10.6	29	97.3	9.0	29y	L653V/L653V	ND	ND
16	5	F	NA	NA	31.2	TF	5.88	13.3	3.75	3.69	13.5	—	P68S/W	ND	ND
17 ^{d,f}	5	F	NA	9.6	75.6	Familial	41.25	12.6	14	138.5	9.0	14y	L653V/L653V	Normal	Normal
18 ^d	5	F	NA	NA	123	Palpitation	52.5	13.3	19	100.2	9.9	19y	L653V/L653V	Enlarged lobe	Normal
19	5	F	NA	NA	84	Viteligo	6.45	17	9.5	6.45	17.5	—	L653V/P68S	ND	ND
20	5	M	NA	NA	133	Familial	6.02	NA	15.0	4.05	ND	—	L653V/W	ND	ND
21	5	M	NA	NA	17	TF	8.83	11.6	6.83	7.7	12.6	—	R450C/W	ND	ND
22	5	M	ND	10.2	51	Familial	31.78	14.07	10.1	5.7 ^b	15.1 ^b	5.33y	L653V/L653V	Normal	Normal
23	5	M	21.3	7.92	0.1	Screening	10.59	14.2	1.66	17.65	14.7	—	L653V/W	ND	Normal
24	5	M	61	8.8	0.7	Screening	91.0	15.8	6.1	0.65 ^b	18.7 ^b	0.7m	L653V/L653V	Low uptake	Normal
25	5	M	NA	NA	96	TF	12.67	14.2	11.0	12.39	13.1	—	L653V/P68S	ND	Normal
26	5	M	NA	NA	28	Familial	4.22	15.9	ND	ND	ND	—	R450C/W	ND	ND
27	5	F	NA	NA	83	TF	7.7	12.4	12.33	8.41	9.3	—	L653V/W	ND	ND
Reference range			<20	>10			0.35–5.0	10.8–18.7		0.35–5.0	10.8–18.7				

Patients 9 and 11 were heterozygous for mutation of TPO gene (C493S).

^aNephrotic syndrome.^bLT4 therapy.^cRefused to take medication.^dLT4 therapy was initiated at last visit.^ePositive TGab at last visit.^fConvulsive disorder.

TT4, total thyroxine; TF, thyroid function; NA, not available; ND, not done.

FIG. 2. TSH and fT4 concentrations as a function of age in subjects heterozygous (left panel) and homozygous (right panel) for *TSHR* gene mutations. Dashed lines represent age-related reference ranges (15).

glands. Eleven subjects were treated with LT4 based on individual physicians' decisions. However, neither clinical evidence of hypothyroidism nor biochemical parameters suggestive of hypothyroidism (elevated creatine phosphokinase or hyperlipidemia) were observed.

Discussion

A high prevalence of *TSHR* mutations was identified among 94 subjects with RTSH. Nicolletti *et al.* (9) reported similar findings among children with nonautoimmune SCH in Italy, but in that study, all of individuals were heterozygous, whereas in the present cohort, 16 subjects had either homozygous or compound heterozygous mutations. Contrary to the Italian cohort, which enrolled only SCH subjects, both SCH and CH children with RTSH characteristics were included in the current study. Moreover, the present cohort included family members of the index cases, which could result in selection bias. A prevalence of 4.3% biallelic *TSHR* mutations was shown among 134 Japanese infants with CH (8), and 12% of both one mutant allele and biallelic mutations were found in 42 subjects with nonautoimmune isolated

hyperthyrotropinemia in Italy; all displayed familial occurrence (12). Camilot *et al.* (13) identified 13 patients with heterozygous mutations (11%) out of 116 children with asymptomatic euthyroid hyperthyrotropinemia. A prevalence of 0.6% for carriers of the *TSHR* mutation W546X was identified in Welsh euthyroid individuals (14).

The high prevalence of *TSHR* mutations within the present cohort is explained by the high occurrence of consanguinity in the population (38%), which lives in the same geographical area, and by the selective enrolment criteria. Furthermore, the identification of only six different mutations is explained by a founder effect in the extended families (10,11). Whereas in the five previously described mutations, functional analysis revealed that these sequence changes result in *TSHR* inactivation (3,10,11) for the novel identified sequence variant c.1347C>T (p.R450C), no functional analysis was performed. Nevertheless, 67 subjects with characteristics of RTSH were negative for *TSHR* mutations, suggesting additional etiologies for nonautoimmune SCH. Therefore, further molecular analysis is indicated to delineate the specific etiologies for SCH in those cases. A nonsignificant trend toward decreasing fT4 levels within the normal ranges for age was

observed in the subjects without *TSHR* mutations, with a few cases having fT4 levels below the reference range.

Patients were found to have SCH following routine thyroid function tests for various conditions that retrospectively were not related to clinical hypothyroidism, and for familial occurrence of abnormal thyroid function tests. Only five infants carrying *TSHR* mutations were detected by the neonatal screening because the Israeli national screening is based on the measure of TT4, and only infants with low TT4 had their TSH analyzed further. Among the patients with CH, only one patient had persistent hypothyroidism. The other 22 patients were diagnosed later due to various complaints or because of familial occurrence of thyroid abnormalities. Those patients with *TSHR* mutations had significantly higher TSH levels compared to those without mutations. However, fT4 levels only differed at the last visit, and they were within the reference range in both groups. When the group with *TSHR* mutations was subdivided into those with one mutant allele and those with a biallelic mutation, the homozygotes were seen to have a more severe phenotype, manifested by significantly higher serum TSH values. Despite this difference in TSH, fT4 levels did not differ between the two subgroups.

In a previous study, it was shown by cross-sectional analysis of 33 affected family members, including parents and grandparents carrying the same two *TSHR* mutations (L653V and P68S), that heterozygous individuals have stable TSH levels that do not require medical therapy (10). In the present study, the long-term outcome over an 11-year period is summarized in individuals identified at 3 days to 21 years as being affected by RTSH by retrospectively analyzing the TSH and fT4 values in each individual. As in the authors' previous report, individuals heterozygous for *TSHR* mutations had stable TSH levels when results from the first and last visit were compared. The trend toward decreasing fT4 values over the years is explained by the normal age-related decline in thyroid hormone levels in childhood (15). In contrast, in the homozygous subgroup, the TSH values showed a significantly increasing trend with concomitant decline in fT4 levels, which in a few subjects reached values below the reference range. These findings indicate incompletely compensated hypothyroidism in individuals carrying biallelic *TSHR* mutations that may necessitate LT4 therapy. There is only one longitudinal study of subjects with *TSHR* mutations, which describes five subjects homozygous for the R450H *TSHR* mutation who presented with congenital hyperthyrotropinemia. Of the five individuals, one developed frank hypothyroidism during adolescence (16), supporting the need for long-term follow-up, especially in homozygotes.

The decision to treat children with SCH is a matter of debate (17–20). The risk of developing overt hypothyroidism in an adult population with SCH has been estimated at 2–4.3% per year, with a higher occurrence in patients with evidence of autoimmune thyroid disease and increased TSH at presentation (17). In children, most of the subjects with SCH remain euthyroid over time, and therefore careful follow-up, rather than empirical treatment, has been suggested (21,22). It is commonly accepted that children with a TSH > 10 mIU/L should be treated, even if the fT4 level is within the reference range, whereas those with a TSH between 4.5 and 10 mIU/L with thyroid autoantibodies should be followed up with repeated thyroid function tests, but not treated (23). This approach was followed, and the target

of treatment was to maintain a serum TSH in the reference range. However, in the present cohort, some subjects with a TSH > 20 mIU/L were not treated, either because the patients refused to take medication or because they were lost to follow-up. Despite the fact that some subjects were not treated for as long as nine years, no pituitary hyperplasia was demonstrated, suggesting that pituitary imaging is not required in this context. Autoimmune thyroid disease concomitant with *TSHR* mutations may lead to overt hypothyroidism (24). This is most likely due to the concomitant thyroid gland destruction by the autoimmune process, which does not occur in RTSH alone. Nevertheless, the benefit of LT4 therapy has been questioned, and some studies have shown no difference in metabolic parameters between treated and untreated subjects (17). This study is the first long-term follow-up of children with nonautoimmune SCH that provides evidence of the clinical courses. SCH in subjects heterozygous for loss-of-function *TSHR* mutations is a stable compensated condition with an appropriately adjusted set point for pituitary–thyroid feedback that does not require replacement therapy. However, in homozygous subjects, incompletely compensated SCH may develop over time, which may necessitate LT4 therapy. In view of the controversy that still exists today regarding LT4 therapy in SCH and the present findings, it is recommended that the decision to initiate therapy be considered on an individual basis, taking into account the benefits and possible side effects.

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