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Research Article

Heterozygous TSHR Variants in Pediatric Idiopathic Subclinical Hypothyroidism: Association with a Variable and Compensated Thyroid Phenotype

Ayşe Yaşar[1], Murat Hakkı Yarar[2], Heves Kırmızıbekmez[1], Fatma Dursun[1]
[1]Department of Pediatric Endocrinology, University of Health Science Umraniye Training and Research Hospital, Istanbul, Turkey
[2]Department of Medical Genetics, University of Health Science Umraniye Training and Research Hospital, Istanbul, Turkey

What is already known on this topic?
Heterozygous TSHR variants are an established genetic cause of nonautoimmune subclinical hypothyroidism in childhood. Available data suggest that many affected individuals exhibit partial TSH resistance with a generally mild and stable clinical course, although long-term outcome data in homogeneous pediatric cohorts remain limited.

What this study adds?
This study includes a homogeneous pediatric cohort and demonstrates a high prevalence (35%) of heterozygous TSHR variants in a well-characterized idiopathic subclinical hypothyroidism cohort and provides long-term longitudinal data. It delineates clinical features associated with treatment necessity and supports the concept that a subset of these patients may exhibit a compensated mild TSH resistance phenotype requiring individualized management.

Abstract
Background: Subclinical hypothyroidism (SH) in childhood is frequently idiopathic and usually follows a benign course. Heterozygous loss-of-function variants in the thyrotropin receptor (TSHR) gene have emerged as a genetic cause of isolated hyperthyrotropinemia; however, data regarding long-term outcomes and management are limited.
Methods: We retrospectively evaluated 51 children (aged 1–18 years) diagnosed with idiopathic SH followed for a mean of 4.9 ± 2.6 years. TSHR gene analysis was performed using targeted next-generation sequencing in patients selected based on neonatal TSH elevation, family history of SH, thyroid gland in situ, or persistence of SH after levothyroxine withdrawal. Clinical, biochemical, ultrasonographic, and treatment outcomes were compared between patients with and without TSHR variants.
Results: Heterozygous TSHR variants were identified in 18 patients (35.2%), including one novel missense variant. Variant-positive patients generally showed a stable, compensated thyroid phenotype without progression to overt hypothyroidism. Levothyroxine therapy was discontinued in 6 of 11 initially treated patients after genetic diagnosis, with sustained biochemical stability. However, five patients required re-initiation of therapy due to rising TSH levels (15.7–27.8 mIU/L) or clinical symptoms. Lower thyroid volume SDS and the presence of additional clinical risk factors, such as small-for-gestational-age birth or developmental delay, were associated with treatment requirement. No patient developed overt hypothyroidism.
Conclusion: Heterozygous TSHR variants appear to contribute to the pathogenesis of pediatric idiopathic SH and are frequently associated with a stable, compensated thyroid phenotype. These findings support a conservative, individualized management strategy rather than routine levothyroxine therapy.
Keywords: Children; idiopathic subclinical hypothyroidism; TSHR heterozygous variants; long-term follow-up

Ayşe Yaşar, MD, Department of Pediatric Endocrinology, University of Health Science Umraniye Training and Research Hospital, Istanbul, Turkey
aseyasar@gmail.com
ORCID ID: 0000-0002-3335-7976

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Introduction
Subclinical hypothyroidism (SH), also referred to as isolated hyperthyrotropinemia, is characterized by elevated serum thyroid-stimulating hormone (TSH) levels with normal free thyroxine (fT4) and free triiodothyronine (fT3) concentrations, with no clinical signs of hypothyroidism. The diagnosis is usually confirmed after at least two independent measurements of TSH (1,2). Subclinical hypothyroidism is stated "idiopathic" in case a certain cause was not identified. Most cases follow a stable course with a low risk of progression (1,3).
Genetic factors play a significant role in SH pathogenesis. Heterozygous variants in the thyrotropin receptor (TSHR) gene are associated with isolated hyperthyrotropinemia (4). These variants have been reported in up to 30% of affected children and are often associated with neonatal TSH elevation and a positive family history (5). Long-term studies suggest that TSHR-related SH may represent a stable, compensated state with a recalibrated hypothalamic–pituitary–thyroid axis, often not requiring levothyroxine (LT4) therapy (5–7).
Loss-of-function TSHR mutations are the most common cause of TSH resistance (RTSH), with a phenotype ranging from severe congenital hypothyroidism (CH) in biallelic cases to mild, nonautoimmune SH in heterozygous individuals (8–11). Reported variant frequencies range from 11% to 29% among cohorts of patients with subclinical hypothyroidism across different populations (8,9,12–14).
This study aimed to evaluate the frequency, clinical characteristics, and long-term outcomes of heterozygous TSHR variants in children with idiopathic SH.

Materials and Methods
Study Design and Population
This retrospective study included 51 children aged 1–18 years diagnosed with idiopathic SH and followed between 2014 and 2024. SH was defined as persistently elevated TSH (>5.5 mIU/L) with normal fT4 and fT3 on at least two measurements (15).
Patients with autoimmune thyroid disease, structural abnormalities, syndromes, prior thyroid surgery, medications affecting thyroid function, obesity-related TSH elevation, follow-up <12 months, or incomplete data were excluded.
Patients were categorized based on clinical presentation and screening history. Those referred through the national newborn screening program or with documented neonatal TSH elevation were initially evaluated for CH; however, only patients who did not fulfill criteria for

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permanent CH after levothyroxine withdrawal were included in the idiopathic SH cohort. Patients diagnosed later in childhood without evidence of neonatal TSH elevation were classified as having acquired SH.

The study was conducted in accordance with the Helsinki II declaration and approved by the local Ethical Committee of our hospital (Approval No: 459, Date: 26/12/2024).Written informed consent for the use of anonymized clinical and genetic data was obtained from the parents or legal guardians of all participants.

Baseline demographic characteristics, including age (categorized for descriptive purposes as 1–3 years [toddler], 3–10 years [childhood], and 10–18 years [adolescence]), sex, and pubertal status, as well as perinatal history (gestational age, birth weight, neonatal TSH elevation) and family history (thyroid disease, consanguinity), were recorded. Height, weight, and body mass index (BMI) were measured at diagnosis and during follow-up visits at 6–12-month intervals and expressed as standard deviation scores (SDS) using national reference data (16). Pubertal staging was assessed according to Tanner criteria (17).

Levothyroxine therapy was initiated in accordance with guideline recommendations in symptomatic patients or in asymptomatic individuals with TSH levels >10 mIU/L (18,19).

Laboratory and Imaging Assessment

Fasting blood samples were obtained between 08:00 and 10:00 a.m. Serum TSH, fT4, fT3, and thyroglobulin (Tg) were measured using an electrochemiluminescence immunoassay on a Cobas 8000 analyser (Roche Diagnostics). Reference ranges were: TSH 0.35–4.2 mIU/L, fT3 2–4.4 ng/L, fT4 0.85–1.70 ng/dL, and Tg 3.5–77 ng/mL.

Ultrasonography was performed by a pediatric radiologist using high-frequency linear transducers (4.8–11 or 5–14 MHz) on Toshiba Aplio 500/300 or Siemens Acuson S3000 systems. Thyroid volume, echogenicity, and nodular structure were evaluated using standardized imaging criteria.

Patient Selection for TSHR Gene Testing

Idiopathic SH was defined after thorough exclusion of all secondary causes. Genetic analyses for *TSHR* was performed in patients who fulfilled at least one of the following criteria:

(i) Neonatal screening history: Persistent elevation of TSH with normal fT4 after withdrawal of LT4 therapy in infants identified by the national newborn screening program for congenital hypothyroidism (withdrawal trial applied when LT4 dose <2 µg/kg/day) (20).

(ii) Family history: Presence of subclinical hypothyroidism in a first-degree relative.

(iii) Thyroid gland in situ: Thyroid gland located in situ with normal or hypoplastic size on ultrasonography.

(iv) Levothyroxine withdrawal evaluation: In patients previously treated for idiopathic SH, thyroid function tests, thyroid autoantibodies, and thyroid ultrasonography were reassessed after LT4 discontinuation. Persistence of SH in two measurements taken ≥2 months apart was considered diagnostic. Re-initiation of LT4 therapy was not based solely on TSH elevation but was considered in the presence of sustained TSH levels >10 mIU/L on repeated measurements and/or clinical symptoms suggestive of hypothyroidism. Asymptomatic patients with stable TSH levels between 5–10 mIU/L and normal fT4 concentrations were monitored longitudinally without treatment. Treatment decisions were made based on overall clinical assessment rather than isolated biochemical findings (1).

Phenotypic Interpretation of Variant-Positive Cases

To improve clinical interpretation, variant-positive patients were further evaluated according to the degree of genotype–phenotype concordance. Cases were pragmatically classified as: (i) solved/likely RTSH phenotype, (ii) indeterminate phenotype, or (iii) unresolved phenotype. This classification was based on longitudinal biochemical profile, family segregation pattern, thyroid imaging findings, neonatal thyroid status, and overall clinical course. The proposed categorization was intended solely as a descriptive clinical framework to aid phenotypic interpretation and should not be considered a formal assessment of pathogenicity, as all identified variants were classified as variants of uncertain significance (VUS).

Genetic Analysis

TSHR gene analysis was performed using a targeted next-generation sequencing (NGS) assay covering all coding exons and exon–intron boundaries. Sequencing was conducted on Illumina platforms, and data were analyzed using a validated bioinformatics pipeline with alignment to the hg38 reference genome. Variants were classified according to American College of Medical Genetics and Genomics (ACMG) guidelines.

The panel also included genes associated with congenital hypothyroidism (DUOX2, DUOXA2, TG, TPO, SLC5A5, IYD, PAX8, NKX2-1). In the initial cohort, eight patients were identified as carrying heterozygous variants in genes other than TSHR (DUOX2, PAX8, and TG) and were excluded to minimize potential oligogenic effects and ensure a genetically homogeneous cohort.

Variants with low analytical reliability, synonymous changes without predicted functional impact, and common benign variants were excluded. All identified TSHR variants were classified as VUS. This approach does not detect large genomic rearrangements or deep intronic variants; therefore, findings were interpreted in conjunction with clinical and biochemical data.

The cohort (n = 51) was stratified based on TSHR sequencing results into variant-positive (Group 1, n = 18) and no detectable TSHR variant (Group 2, n = 33). Comparative analyses were performed between the two groups in terms of clinical characteristics and biochemical parameters. Group 1 was also assigned into subgroups based on initial therapeutic approach into treated (Group 1a, n = 11) and untreated patients (Group 1b, n = 7).

Statistical Analyses

Statistical analyses were performed using IBM SPSS Statistics for Windows, Version 22.0 (IBM Corp., Armonk, NY, USA). The normality of data distribution was assessed using the Shapiro–Wilk test. Continuous variables were expressed as mean ± standard deviation (SD) or median (interquartile range, IQR), as appropriate. Categorical variables were analyzed using the chi-square test. Comparisons between groups were performed using the Mann–Whitney U test. All tests were two-tailed, and a p-value <0.05 was considered statistically significant.

Results

Study population

A total of 51 patients with idiopathic SH were included (29 girls, 22 boys). The mean age at diagnosis was 7.9 ± 4.1 years (range: 1.2–16.6), and the mean follow-up duration was 4.9 ± 2.6 years**.** At presentation, 17 patients were at adolescence, 26 were in childhood age, and 8 were toddlers. At presentation 30 patients (~60%) were at prepubertal stage. Most patients exhibited a standard perinatal history, with 70% being born at term and 80% exhibiting an appropriate birth weight for gestational age.

Sixteen patients were referred through the national newborn screening program and were considered to have congenital onset, whereas 35 were classified as having acquired SH based on thyroid function abnormalities detected during childhood, family history or nonspecific symptoms. A history of neonatal TSH elevation was identified in 24 patients, of whom 16 were detected through the screening program and eight patients were identified retrospectively during follow-up.

Patients referred through the screening program were initially treated with levothyroxine, and withdrawal trials were performed during follow-up. After withdrawal, therapy was re-initiated in four patients, who were subsequently classified as having permanent CH, while the remaining patients were considered to have idiopathic SH.

Among the 35 patients initially classified as having acquired SH, 21 were followed without treatment, while 14 had received levothyroxine at presentation. Following withdrawal trials, therapy was re-initiated in six patients. TSHR variants were identified in three of these patients, and the reasons for re-initiation are detailed in the Discussion section; in the remaining three, therapy was re-initiated due to serum TSH

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levels >10 mIU/L.
A first-degree family history of SH was present in 38 patients, and parental consanguinity was identified in nine cases.

Genetic Analysis of the TSHR Gene

The clinical characteristics of the 18 patients (35.2%) carrying a *TSHR* variant are summarized in Table-1. All variants were heterozygous. A total of nine distinct missense variants were detected in these patients. Eight variants had been previously reported in the literature (p. Asp474Glu, p. Thr62Ala, p. Pro162Ala, p. Gly245Ser, p. Pro68Ser, p. Glu506Lys, p. Arg519His, p. Ala593Val), whereas one (p. Ile117Met) was novel. The variants were distributed across the TSHR gene without evidence of a defined hot-spot region. Bioinformatic predictions indicated loss-of-function effects for all missense variants. The most frequent variant was p. (Thr62Ala), identified in five patients. Among the 18 variant-positive patients, 11 had a retrospectively reported family history of elevated TSH, whereas seven had no documented family history.

Clinical Course of Variant-Positive Patients

Four of the 18 variant-positive patients were referred through newborn screening (cut off: 5.5 mU/L); the remaining cases were evaluated because of a family history or clinical suspicion. Although baseline TSH levels were higher in patients who initially received L-T4 therapy (Group 1a, n = 11; 10.6±4.71 mIU/L) compared with untreated patients (Group 1b, n = 7; 7.39±1.86 mIU/L), this difference was not statistically significant (p = 0.181).

Following identification of the TSHR variant, treatment withdrawal was attempted in all Group 1a patients under close biochemical and clinical surveillance. Five maintained a stable SH phenotype without further intervention. In five patients (No 2c, 3c, 7, 8, and 12), LT4 therapy was re-initiated after at least 6 months of follow-up due to persistently elevated TSH levels (mean 21.1 ± 4.58 mIU/L) confirmed on repeated measurements and/or the development of clinical or structural features suggestive of impaired thyroid function. Several of these patients also exhibited additional clinical features, including thyroid hypoplasia, hyperthyrotropinemia related hyperprolactinemia and oligomenorrhea, a history of small for gestational age (SGA), developmental delay and obesity. In patient No 13, although LT4 therapy had been initiated at diagnosis, follow-up data were insufficient to clearly determine treatment status during follow-up, and therefore this patient was not included in the re-initiation group. All patients in Group 1b remained off therapy throughout follow-up, with TSH values consistent with euthyroidism or SH. In addition, anthropometric measures and thyroid function tests did not differ significantly between Groups 1a and 1b at diagnosis or at the final evaluation. Thyroid volume SDS was significantly lower in Group 1a compared with Group 2 (p = 0.023).

Among the 18 variant-positive patients, genotype–phenotype concordance showed marked heterogeneity. The multigenerational family cluster carrying the p.Thr62Ala variant (patients 2c–6c) was considered the subgroup most strongly consistent with a "solved/likely RTSH phenotype" because of familial clustering and persistent nonautoimmune hyperthyrotropinemia. In contrast, several other cases were interpreted as having "indeterminate" or "unresolved" phenotypes due to spontaneous normalization of thyroid function, absence of segregation analysis, or atypical clinical findings not fully explained by heterozygous TSHR variants alone. In particular, patients who became euthyroid during follow-up (patients 9a, 9b, and 10) were classified as having an "unresolved phenotype," since spontaneous biochemical normalization is not fully compatible with persistent genetically determined partial TSH resistance. Likewise, the two patients with thyroid hypoplasia were considered clinically atypical and interpreted as being closer to an "indeterminate phenotype," suggesting the possible contribution of additional genetic or non-genetic mechanisms beyond isolated heterozygous TSHR variation.

The findings suggest that thyroid function remains stable in a substantial proportion of individuals with heterozygous TSHR variants, and the need for treatment varies according to the clinical characteristics.

Comparison with Variant-Negative and Variant-Positive Patients

Clinical and laboratory characteristics of variant-positive patients are presented in Table-2. Overall, anthropometric measures and thyroid function remained stable from diagnosis to the final follow-up, and no clinically significant progression in thyroid volume was observed. Comparisons between the groups revealed generally similar biochemical and anthropometric profiles, indicating that *TSHR* variants did not exert a significant impact on thyroid function or growth parameters. At the last follow-up, TSH levels were significantly higher in Group 1. Although the proportion of patients continuing LT4 therapy was greater in the variant-positive group, this difference did not reach statistical significance (Table-2). Although most demographic and clinical characteristics were comparable between groups, several significant differences were observed in patients carrying TSHR variants. The frequency of persistent neonatal TSH elevation was significantly lower in the variant-positive group (p = 0.001). At the final follow-up, SH was significantly more common among variant positive patients compared with variant-negative individuals (p = 0.015). In addition, a family history of thyroid disease was present in all patients with *TSHR* variants and was significantly more frequent than in the variant-negative group (p = 0.030) (Table-3).

Discussion

This study provides comprehensive data on the prevalence, phenotype, and long-term clinical course of heterozygous *TSHR* variants in children with idiopathic SH. The detection of a 35% variant frequency, including three previously unreported variants, highlights the considerable genetic heterogeneity underlying TSHR-related thyroid dysfunction. The frequent occurrence of neonatal TSH elevation and a family history of thyroid disease, together with the predominance of diagnosis during childhood, suggests that TSHR-related mild thyroid dysfunction tends to become clinically apparent in the pediatric age group.

Previous studies have reported widely variable frequencies of TSHR loss-of-function variants, ranging from 4% to 52%. These cohorts consistently highlighted the prominence of a positive family history and the frequent identification of cases through newborn screening (5,8, 12,13,21,22). In a cohort of 111 children with SH, Vigone et al. (2017, Italy) identified 17 distinct TSHR variants in 34 patients, eight of which were novel; notably, 17 patients were detected through newborn screening and 27 had a family history of SH (8). Similarly, Tanenbaum-Rakover et al. (2015, Israel) reported that five of 27 patients carrying TSHR variants had abnormal newborn screening results (6). In another study, Calebiro et al. (2012, Italy) identified TSHR variants in 12% of children with non-autoimmune SH (21).

Across these reports, most affected individuals were diagnosed in early to mid-childhood, particularly between 6 and 12 years of age; this pattern indicates that TSHR-related mild thyroid dysfunction is typically recognized in the pediatric age group. These studies were conducted in heterogeneous cohorts that included various SH subtypes and diverse TSHR genotypic profiles. In contrast, our study focused exclusively on children with idiopathic SH who were simple heterozygous carriers of TSHR variants. Consequently, our cohort represents a more homogeneous and clinically refined population compared with many previously published series. The 35% variant prevalence we observed falls within the upper range of reported frequencies, likely to reflect this methodological specificity. Consistent with previous reports, we found that TSHR-related SH most commonly manifests during childhood, underscoring the importance of early recognition and long-term follow-up.

Interestingly, the rate of persistent neonatal TSH elevation was lower in the variant-positive group. This finding is not fully consistent with the expected congenital presentation. However, a direct causal relationship cannot be established due to the lack of segregation and functional validation. Therefore, these variants should be interpreted with caution and may be considered genetic modifiers contributing to phenotypic variability rather than primary disease-causing variants.

The need for treatment in TSHR mutation–related SH remains a matter of debate (23). In children with genetically confirmed RTSH, particularly those older than 3 years who are asymptomatic and exhibit normal growth, treatment decisions should not rely solely on TSH levels (24). This condition is considered a compensated dysfunction arising from the adaptation of the hypothalamic–pituitary–thyroid axis to a new equilibrium, and pharmacological therapy is generally not recommended in most cases. In our cohort, L-T4 therapy was discontinued in 45% of treated patients (5/11) following the identification of a TSHR variant.

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Among patients in whom treatment was either not initiated or subsequently withdrawn, TSH levels ranged between 4.42 and 19.1 mU/ml, and no clinical signs of hypothyroidism were observed during two years of follow-up, even in the patient with the highest TSH value (patient 4c). Although previous studies have reported discontinuation rates of up to 70% after reassessment, with TSH levels typically remaining within the 5–10 mU/ml range (6,8), our rates were lower. This discrepancy may be attributable to the higher baseline TSH levels observed in our cohort.

As highlighted by Kara et al., variability in the TSH set point in RTSH indicates that treatment decisions should be guided by clinical findings rather than biochemical thresholds alone (25). Accordingly, re-initiation of L-T4 therapy in our cohort was based on an individualized assessment, considering features such as SGA history, hyperprolactinemia-related oligomenorrhea, obesity, and, in one patient, thyroid hypoplasia with marked TSH elevation (27.8 mIU/L).

Radetti et al. reported that patients in whom L-T4 therapy was re-initiated were most often either compound heterozygotes or single heterozygous individuals with additional risk factors for thyroid dysfunction, such as being SGA (26). Similarly, Vigone et al. emphasized that L-T4 therapy may also be considered in single heterozygous patients belonging to special risk groups, including those born preterm, SGA, after multiple pregnancies, or conceived through assisted reproductive techniques (8). However, even in light of these studies, the presence of SGA alone should not be considered a definitive indication for treatment. Among the five cousins carrying the same variant in our cohort, two had a history of SGA and similar biochemical findings, suggesting a possible association between the variant and the phenotype. In particular, LT4 therapy in patient 2c was re-initiated not only because of the history of SGA, but also due to a decline in height SDS observed during follow-up (from −2.09 to −2.48). The subsequent improvement in height SDS after treatment (−2.02) further supported this approach. In contrast, the remaining three variant-positive individuals remained clinically and biochemically stable; therefore, conservative follow-up was preferred.

Although thyroid hypoplasia is not a typical feature of SH associated with TSHR variants, this finding was observed in two patients in our cohort. Patient No 7 was referred through the newborn screening program with a preliminary diagnosis of CH and was initiated on levothyroxine therapy. Following a trial of treatment withdrawal, L-T4 therapy was re-initiated due to persistent elevation of TSH levels. No variants were identified in other genes associated with CH. Segregation analysis could be performed only in this patient and demonstrated the presence of the same TSHR variant in the mother; this finding was considered supportive of a potential clinical effect of the variant. Patient No 8, in contrast, was followed as having acquired subclinical hypothyroidism. L-T4 therapy was initially initiated but discontinued after the identification of a TSHR variant. During follow-up, the development of oligomenorrhea and hyperprolactinemia prompted the re-initiation of levothyroxine. Following normalization of TSH levels after treatment, prolactin levels also normalized and regular menstrual cycles were restored.

In our cohort, obesity was present in one of the patients in whom LT4 therapy was re-initiated. In this case, obesity had already been present prior to treatment withdrawal and did not newly develop during follow-up. It is well established that obesity in children is associated with mild elevations in TSH levels, which are generally considered adaptive and reversible. However, in the context of genetically confirmed RTSH, the coexistence of obesity may complicate the interpretation of thyroid function and, in selected cases, may raise concern for relative tissue-level thyroid hormone insufficiency. In this patient, the presence of relatively high TSH levels (≈19 mIU/L), together with obesity as an additional metabolic risk factor, contributed to the clinical decision to continue treatment.

These observations indicate that heterozygous TSHR variants may present with a broader and more heterogeneous clinical spectrum than traditionally expected. Previous studies have shown that even carriers of the same TSHR variant within the same family can exhibit markedly different TSH levels, along with notable fluctuations over time within the same individual. Collectively, these suggests that treatment decisions should not be based solely on the presence of a genetic variant but rather should be guided by the individual clinical course and biochemical characteristics of each patient. Environmental factors (e.g. iodine intake, acquired thyroid disorders, and drugs) or other genetic modifiers (e.g. polygenic inheritance, polymorphisms in thyroid hormone pathway genes, and epigenetic factors) are probably responsible for such variability (27). Therefore, the atypical morphological and biochemical features observed in our patients may not be solely attributable to a single genetic variant. Further molecular and functional studies are needed to better elucidate these mechanisms.

Tenenbaum et al. reported in their long-term follow-up of 27 children with TSHR-related SH and CH that, in heterozygous cases, the hypothalamic–pituitary–thyroid feedback set point remains appropriately maintained, resulting in a stable and mild clinical course (6). Similarly, Calebiro et al. (21) described a maximum TSH level of 17 mIU/L in a 2-month-old infant, whereas in our cohort the highest TSH concentration was 27.8 mIU/L, observed in a 5-year-old girl. This finding suggests that the phenotypic expression of *TSHR* variants may vary across age groups and that a compensated state can be preserved even at higher TSH levels in some patients.

In patients heterozygous for TSHR mutations, SH is generally considered a compensated thyroid dysfunction with an appropriately adjusted pituitary–thyroid set point and does not usually require treatment (1). However, it may progress to a decompensated state, particularly in the presence of concomitant thyroid disease (7,9). Vigone et al. (8) reported that discontinuation of L-T4 was not associated with adverse clinical or biochemical outcomes.

Our findings are largely consistent with the existing literature. Among variant-positive patients, anthropometric measures, thyroid function tests, and thyroid volume remained stable from diagnosis to the final assessment, and none of the untreated or treatment-discontinued children developed clinical hypothyroidism. However, the re-emergence of treatment requirements in some patients particularly those with higher TSH levels, lower thyroid volume SDS, a history of SGA birth, or additional clinical risk factors suggests that a treatment-free approach may not be equally safe for all individuals. Therefore, treatment decisions in children carrying heterozygous TSHR variants should be carefully individualized, incorporating thyroid volume and the overall clinical risk profile into a comprehensive assessment.

All identified variants were classified as VUS according to ACMG criteria, and no definitive conclusions regarding their pathogenicity can be drawn. Accordingly, identification of a heterozygous TSHR variant should not automatically be considered equivalent to a definitive molecular diagnosis of RTSH, particularly in the absence of segregation, functional, or longitudinal supportive evidence.

The absence of segregation analysis and functional validation, as well as the lack of formal exclusion of macro-TSH, constitute important limitations of the study and restrict causal inference; however, the observed phenotypic consistency supports a potential contributory role. In addition, iodine status was not assessed biochemically; however, no evidence suggestive of iodine imbalance was identified based on clinical history, and the study population was derived from a region considered iodine sufficient. Nevertheless, the absence of objective iodine measurements should also be acknowledged as a significant limitation.

Despite these limitations, the prolonged follow-up and consistent biochemical stability observed in variant-positive individuals provide clinically meaningful evidence supporting a compensated, mild TSH resistance phenotype rather than progressive thyroid dysfunction.

Conclusion:

Heterozygous TSHR variants were frequently identified in children with idiopathic SH and were associated with a generally stable and compensated thyroid phenotype in a subset of patients. However, variant detection alone was insufficient to establish definitive RTSH in all individuals. While most patients could be managed conservatively, selected cases required individualized treatment decisions based on clinical course and biochemical severity.

Conflict of interest

The authors have no conflict of interests to declare.

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Authorship Contributions
Concept: AY, FD, Design: AY, FD, Data collection or processing: AY, FD, MHY, Analysis or interpretation: AY, FD, MHY, Literature Search: AY, FD, Writing: AY, FD, HK

Table Legends
Table 1: Clinical, genetic, and thyroid-related characteristics of patients carrying heterozygous TSHR variants
Table 2. Comparison of clinical, biochemical, and diagnostic characteristics between TSHR variant-positive and variant-negative patients at baseline and final follow-up
Table 3. Comparison of demographic, clinical, and biochemical characteristics between the groups.

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Table 1: Clinical, genetic, and thyroid-related characteristics of patients carrying heterozygous TSHR variants

No.	Sex-Age at diagnosis (year)	Follow-up duration (year)	Gen/Transcript/Genomic loc.(hg38)	HGVS /ACMG	Baseline TSH* (mU/ml)	LT4 at diagnosis	Thyroid ultrasound	Highest TSH* (mU/ml) during follow-up	Clinical feature	LT4 during follow-up	Final thyroid status

UNCORRECTED PROOF

1a	F-6.3y	3y	TSHR NM_000369.5 chr14:81143480 C>A exon 10	c.1422C>A p.Asp474Glu missense VUS	6.58	No	Normal	6.67	None	No	SH
1b	F-5.11y	10y	TSHR NM_000369.5 chr14:81143480 C>A exon 10	c.1422C>A p.Asp474Glu missense VUS	10.9	Yes	Normal	8.47	None	No	SH
2[c]	F-2.6y	4.3y	TSHR NM_000369.5 chr14:81062161 A>G exon 2	c.184A>G p.Thr62Ala missense VUS	14.8	Yes	Normal	20.4	Growth delay (SGA)	Yes	SH
3[c]	M-8.1y	9y	TSHR NM_000369.5 chr14:81062161 A>G exon 2	c.184A>G p.Thr62Ala missense VUS	13.6	Yes	Normal	15.7	None (preterm SGA)	Yes	SH
4[c]	F-5.3y	7y	TSHR NM_000369.5 chr14:81062161 A>G exon 2	c.184A>G p.Thr62Ala missense VUS	15.6	Yes	Normal	19.1	None	No	SH
5[c]	F-9.9y	4y	TSHR NM_000369.5 chr14:81062161 A>G exon 2	c.184A>G p.Thr62Ala missense VUS	13.6	Yes	Normal	7.35	None	No	SH
6[c]	F-13.1y	4y	TSHR NM_000369.5 chr14:81062161 A>G exon 2	c.184A>G p.Thr62Ala missense VUS	12.9	Yes	Normal	7.52	None	No	SH
7	F-3.11y	8y	TSHR NM_000369.5 chr14:81092547 C>G exon 6 rs121908863	c.484C>G p.Pro162Ala missense VUS	15.6	Yes	Hypoplastic	27.8	None	Yes	SH
8	F-15.11y	4y	TSHR NM_000369.5 chr14:81087987 A>G exon 4	**c.351A>G p.Ile117Met** missense VUS	9.56	Yes	Hypoplastic	25.5	Hyperprolactinemia/Oligomenorrhea	Yes	SH
9a	F-12.8y	4y	TSHR NM_000369.5 chr14:81139719 G>A exon 9 rs189506473	c.733G>A p.Gly245Ser missense VUS	10.9	No	Normal	4.42	None	No	Euthyroid
9b	F-11.2y	7y	TSHR NM_000369.5 chr14:81139719 G>A exon 9 rs189506473	c.733G>A p.Gly245Ser missense VUS	14.3	No	Normal	4.91	None	No	Euthyroid

UNCORRECTED PROOF

10	M-9.11y	6y	TSHR NM_000369.5 chr14:81062179 C>T exon 2 rs142063461	c.202C>T p.Pro68Ser missense VUS	11.1	No	Normal	4.95	None	No	Euthyroid
11	F-12y	7y	TSHR NM_000369.5 chr14:81143574 G>A exon 10 rs762048531	c.1516G>A p.Glu506Lys missense VUS	15.2	Yes	Normal	7.43	None	No	SH
12	M-12.5y	4y	TSHR NM_000369.5 chr14:81143836 C>T exon 10	c.1778C>T p.Ala593Val missense VUS	9.53	Yes	Normal	19	Obesity	Yes	SH
13	F -15.1y	4y	TSHR NM_000369.5 chr14:81092547 C>G exon 6 rs121908863	c.484C>G p.Pro162Ala missense VUS	10.5	Yes	Normal	18.5	None	-	SH
14	F-11.9y	4y	TSHR NM_000369.5 chr14:81143614 G>A exon 10 rs780018604	c.1556G>A p.Arg519His missense VUS	10.2	No	Normal	8.62	None	No	SH
15a	M-6.9y	2y	TSHR NM_000369.5 chr14:81062179 C>T exon 2 rs142063461	c.202C>T p.Pro68Ser missense VUS	9.1	No	Normal	10.1	Twin/None	No	SH
15b	M-6.9y	2y	TSHR NM_000369.5 chr14:81062179 C>T exon 2 rs142063461	c.202C>T p.Pro68Ser missense VUS	9.1	No	Normal	10.1	Twin/None	No	SH

Novel variants not reported in gnomAD v4.1.0 are indicated in bold.
Suffixes: "a", index patient; "b", sibling; "c", cousin from the same extended family.
Abbreviations: SH, subclinical hypothyroidism; LT4, levothyroxine; SGA, small for gestational age; M: male; F: female.
*Highest TSH value recorded baseline and during follow-up.

Table 2. Comparison of clinical, biochemical, and diagnostic characteristics between TSHR variant-positive and variant-negative patients at baseline and final follow-up

Parameter	**Group 1 (TSHR variant +) Mean ± SD n=18**	**Group 2 (TSHR variant -) Mean ± SD n=33**	**p value**
Presentation			
TSH-1 (0,66-4,75 mIU/L)	8.90 ± 3.10	9.97±3.34	0.983
fT4-1 (0,78-1,31 ng/dL)	1.20 ± 0.19	1.26±0.10	0.405
TSH-2 (0,66-4,75 mIU//L)	9.71 ± 2.48	9.47±2.84	0.239
fT4-2 (0,78-1,31 ng/dL)	1.20 ± 0.20	1.26±0.16	0.413
TG (3,5-77 ng/mL)	32.5 ± 28.40	50.5±42.7	0.186

UNCORRECTED PROOF

Weight SDS		0.06 ± 1.27	-1.73±4.86	0.884
Height SDS		-0.03 ± 1.27	0.08±1.19	0.598
BMI SDS		0.10 ± 1.08	-0.91±1.82	0.838
Thyroid volume SDS		-0.47 ± 1.09	-0.22±0.79	0.331
Last visit				
TSH (0,66-4,75 mIU/L)		8.29 ± 3.87	5.97±3.28	**0.007**
fT4 (0,78-1,31 ng/dL)		1.21 ± 0.18	1.17±0.17	0.076
fT3 (2,56-5,01 ng/dL)		4.29 ± 0.79	4.41±0.43	0.855
Weight SDS		-0.04 ± 1.45	-0.79±0.48	0.884
Height SDS		-0.12 ± 1.96	-0.08±0.89	0.598
BMI SDS		0.10 ± 1.03	-0.68±0.77	0.838
LT4 n (%)	Yes	5(27.7)	7(21.2)	0.181
	No	13(72.2)	26(78.8)	
Diagnosis n (%)	CH	4(22.2)	12(36.3)	0.298
	ASH	14(77.7)	21(63.6)	

Continuous variables were compared using the Mann–Whitney U test; categorical variables were compared using the chi-square test
TSH-1 and fT4-1 represent thyroid function tests at diagnosis (baseline); TSH-2 and fT4-2 represent values at the last follow-up evaluation.
TSH: thyroid stimulating hormone; fT4: free thyroxine; fT3: free Triiodothyronine; TG: thyroglobulin; BMI: body mass index; SDS: standard deviation score; CH: congenital hypothyroidism; ASH: acquired subclinical hypothyroidism.
Bold values indicate statistical significance ($p<0.05$)

Table 3. Comparison of demographic, clinical, and biochemical characteristics between the groups.

Parameter	Category	Group 1 n (%)	Group 2 n (%)	p value
Sex	Female / Male	13 (72.2) / 5 (27.8)	16 (48.5) / 17 (51.5)	0.102
Gestational age	<38 / 38–42 weeks	6 (40)/ 9 (60)	5 (16.1) / 26 (83.9)	0.219
Birth weight	SGA / AGA	2 (13.3)/ 13 (86.7)	2 (6.45) / 29 (93.5)	0.357
Consanguinity	Yes / No	6 (37.5) / 10 (62.5)	3 (11.5) / 23 (88.5)	0.086
Neonatal TSH elevation (persistent)	Yes/ No/Unknown	4 (22)/ 10 (56)/ 4 (22)	20 (61) / 4 (12) / 9 (27)	**0.001***
Pubertal status	Pubertal / Prepubertal	10 (55.6) / 8 (44.4)	11 (33.3) / 22 (66.7)	0.123
Age at diagnosis	Adolescence / Childhood / Toddler	8 (44.4) / 9 (50.0) / 1(5.6)	9 (27.3) / 17 (51.5) / 7 (21.2)	0.240
Final thyroid status	SH / Euthyroid	15 (83.3) / 3 (16.7)	16 (48.5) / 17 (51.5)	**0.015**
Family history of thyroid disease	Yes / No	18 (100) / 0 (0)	20 (74) / 7 (26)	**0.030***

Categorical variables were analyzed using the Chi-square test TSH: thyroid stimulating hormone; SGA: small for gestational age; AGA: appropriate for gestational age; SH: subclinical hypothyroidism
Bold values indicate statistical significance ($p<0.05$).
Neonatal TSH elevation data were obtained from medical records when available and, in some cases, from parental reports. Persistent elevation was defined as TSH levels above the newborn screening cutoff confirmed on repeat testing and/or requiring initiation of L-T4 therapy during infancy.

UNCORRECTED PROOF