Atherosclerosis 415 (2026) 120672

Contents lists available at ScienceDirect

Atherosclerosis

journal homepage: www.elsevier.com/locate/atherosclerosis

# *LDLR* and *APOB* pathogenic variants predict discordant TSH effect on LDL-C

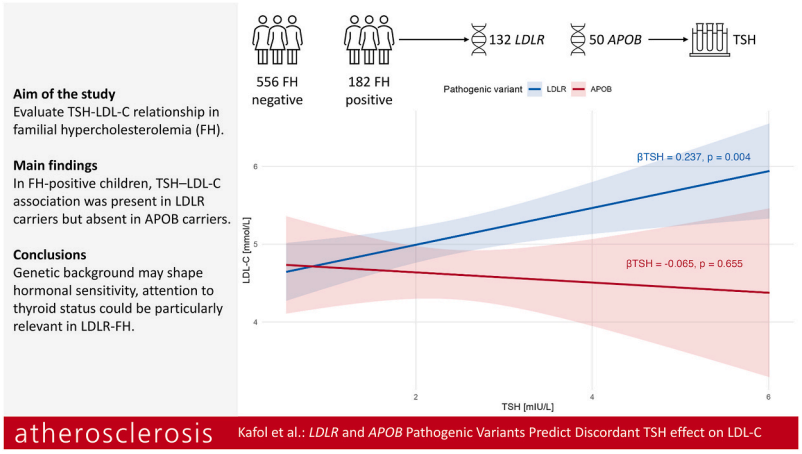
Jan Kafol [a,b], Jaka Sikonja [a,c], Matej Mlinaric [a,d], Barbara Cugalj Kern [a,e], Mihaela Pricop-Jeckstadt [f], Ana Drole Torkar [a,d], Tevz Gorjanc [a,d], Andraž Petek [a], Andreja Lakner [a], Jernej Kovac [a,e], Tadej Battelino [a,d], Urh Groselj [a,d,*]

[a] Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia
[b] Department of Vascular Diseases, Division of Internal Medicine, University Medical Centre Ljubljana, Ljubljana, Slovenia
[c] Department of Endocrinology, Diabetes and Metabolic Diseases, Division of Internal Medicine, University Medical Centre Ljubljana, Ljubljana, Slovenia
[d] Department of Endocrinology, Diabetes, and Metabolic Diseases, University Children's Hospital, University Medical Centre Ljubljana, Ljubljana, Slovenia
[e] Clinical Institute of Special Laboratory Diagnostics, University Children's Hospital, University Medical Centre Ljubljana, Ljubljana, Slovenia
[f] Department of Applied Mathematics, University Politehnica of Bucharest, Bucharest, Romania

## HIGHLIGHTS

- TSH–lipid associations varied by familial hypercholesterolemia status.
- In FH-positive children, TSH showed modest links with LDL-related measures.
- No clear TSH–LDL-C pattern was seen in children with APOB variants.
- Genetic background may influence thyroid–lipid relationships in FH.

## GRAPHICAL ABSTRACT




## ARTICLE INFO

*Keywords:*
TSH
Low-density lipoprotein cholesterol
Familial hypercholesterolemia
*LDLR*
*APOB*
Subclinical hypothyroidism

## ABSTRACT

*Background and aims:* Thyroid hormones regulate lipoprotein metabolism—primarily by up-regulating the LDL receptor. Whether TSH relates to LDL-C in hypercholesterolemic children, and whether this depends on familial hypercholesterolemia (FH) status or the underlying defective gene, is uncertain. We evaluated TSH–lipid associations in prepubertal children and tested effect modification by FH status and, within FH, by gene with a pathogenic variant (*LDLR* vs *APOB*).
*Methods:* We performed a cross-sectional study of prepubertal children referred to the Slovenian national tertiary center through the universal FH screening program or cascade screening. Eligibility required concurrent TSH and

* Corresponding author. Department of Endocrinology, Diabetes, and Metabolism, University Children's Hospital Ljubljana, Department of Pediatrics, Faculty of Medicine, University of Ljubljana, Bohoriceva 20, 1000, Ljubljana, Slovenia.
*E-mail address:* urh.groselj@kclj.si (U. Groselj).

https://doi.org/10.1016/j.atherosclerosis.2026.120672
Received 6 December 2025; Received in revised form 29 January 2026; Accepted 4 February 2026
Available online 9 February 2026

fasting lipid measurement and completed genetic testing (pathogenic/likely pathogenic variants in *LDLR/APOB/PCSK9* vs polygenic hypercholesterolemia).
*Results:* Among 738 children, 182 (24.7%) were FH-positive (*LDLR* 132; *APOB* 50). In the pooled cohort, TSH did not correlate with age or lipids (all $p \geq 0.050$). After sex stratification, TSH correlated with triglycerides only in males ($\rho = 0.156$; $p = 0.012$). In FH-positive children, TSH correlated with total cholesterol, LDL-cholesterol, and ApoB ($\rho \sim 0.184$–$0.207$; all $p < 0.050$), with no associations in FH-negative children. Interaction testing confirmed effect modification by FH (TSH × FH $\beta = 0.141$ mmol/L per mIU/L, $p = 0.023$). Within FH-positive children, a positive TSH–LDL-C slope was seen in *LDLR* carriers ($\beta = 0.237$, $p = 0.004$) but not in *APOB* carriers ($\beta = -0.065$, $p = 0.655$).
*Conclusions:* TSH was positively associated with LDL-C only in FH due to *LDLR* variants, not in *APOB* carriers. These findings suggest that genetic background may shape hormonal sensitivity, and that attention to thyroid status could be particularly relevant in *LDLR*-FH.

## 1. Introduction

Hypercholesterolemia is a key modifiable risk factor for atherosclerotic cardiovascular disease [1]. While monogenic disorders—most notably familial hypercholesterolemia (FH)—account for a substantial proportion of severe low-density lipoprotein cholesterol (LDL-C) elevations, secondary causes are common and clinically relevant [2–4]. Among secondary causes, hypothyroidism is common and easily treatable [5].

Thyroid hormones regulate multiple steps of lipoprotein metabolism. Triiodothyronine (T3) up-regulates hepatic low-density lipoprotein receptor (LDLR) expression and promotes LDL-C clearance; hypothyroidism therefore reduces LDLR activity and increases total cholesterol (TC) and LDL-C [6,7]. In adult populations, higher TSH levels within the reference range have also been associated with higher circulating lipid levels, even after adjustment for thyroid hormone concentrations [8]. Additional mechanisms include altered cholesterol synthesis and bile acid turnover, reduced lipoprotein lipase activity with impaired triglyceride catabolism, and changes in apolipoprotein metabolism that can alter high-density lipoprotein cholesterol (HDL-C) [9].

Subclinical hypothyroidism in children—elevated thyroid-stimulating hormone (TSH) with normal free thyroxine (T4)—is fairly common and most often due to Hashimoto thyroiditis [10]. Pediatric data show that subclinical hypothyroidism is linked to a pro-atherogenic lipid pattern: higher TC and LDL-C, with HDL-C often unchanged or lower, and occasional triglyceride elevations; however, evidence for vascular structural changes is limited and based on small cohorts. These abnormalities worsen with overt hypothyroidism and often improve with levothyroxine [11,12].

Evidence in children linking thyroid status to dyslipidemia is modest, and almost no data exist in the context of FH—the most common monogenic cause of high LDL-C in childhood. In particular, it is unclear whether circulating TSH tracks with LDL-C in prepubertal hypercholesterolemic children, whether any association differs between FH-positive and FH-negative groups, and whether it varies by the defective gene underlying FH (most commonly *LDLR*, less often *APOB* and *PCSK9*). In this article, we sought to (i) describe the relationship between TSH and lipid profile in prepubertal children with hypercholesterolemia without clinically overt hypothyroidism; (ii) test whether the TSH–LDL-C association differs by FH status; and (iii) explore heterogeneity among FH-positive children by comparing carriers of *LDLR* versus *APOB* pathogenic variants. An overview of the study design and main findings is provided in the graphical abstract (Fig. 1).

## 2. Materials and methods

The study was approved by the National Medical Ethics Committee of Slovenia (0120-14/2017/5; 0120-100/2019/5) and conducted in accordance with the Declaration of Helsinki and STROBE reporting guidelines. Written informed consent for genetic testing was obtained from parents or legal guardians.

In this retrospective cross-sectional study, we included prepubertal children aged ≤9 years who were referred to the University Children's Hospital Ljubljana (UCH-LJ) between May 14, 2002 and October 12,

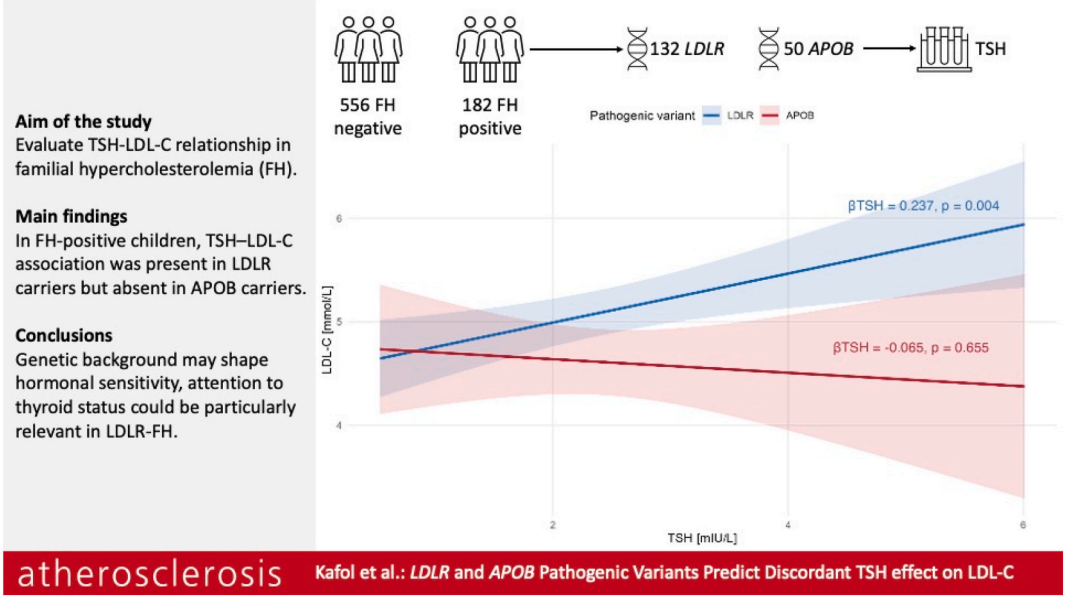



**Fig. 1.** Graphical abstract. Gene-specific association between TSH and LDL-C in prepubertal children with hypercholesterolemia.

2022 for hypercholesterolemia and underwent genetic testing. Prepubertal children were included to minimize the potential influence of hormonal changes associated with puberty on lipid profiles and allowing for a more accurate assessment of the specific effects of thyroid hormones on lipid metabolism. Participants with TSH ≥10 mIU/L were excluded to restrict the cohort to mild subclinical thyroid dysfunction and to avoid confounding from thyroid states commonly considered for treatment. This approach was consistent with the primary aim of the study, which was to evaluate the role of subclinical hypothyroidism and variation in TSH within the normal and mildly elevated range on lipid profiles, rather than more advanced thyroid dysfunction, for which data in this context remain limited [13].

Participants were identified from three sources: (i) the Slovenian nationwide universal screening program for hypercholesterolemia in preschool children [14]; (ii) siblings identified through cascade screening of a proband with genetically confirmed FH; and (iii) other children/adolescents referred for suspected dyslipidemia outside the screening program.

Inclusion criteria:

- age at first thyroid function measurement ≤9 years;
- hypercholesterolemia with completed genetic testing;
- concurrent measurement of lipid profile (TC, HDL-C, LDL-C, triglycerides) and TSH.

Exclusion criteria:

- use of lipid-lowering therapy at the time of TSH measurement;
- TSH ≥10 mIU/L;
- homozygous FH or other rare dyslipidemias;
- genetic result classified as a variant of unknown significance (VUS).

The stepwise inclusion process is shown in Fig. 2. Of the 738 included children, 693 (93.9%) were identified through the universal preschool familial hypercholesterolemia screening program, 38 (5.1%) through cascade testing of relatives, and 7 (0.9%) through other referrals for hypercholesterolemia evaluation.

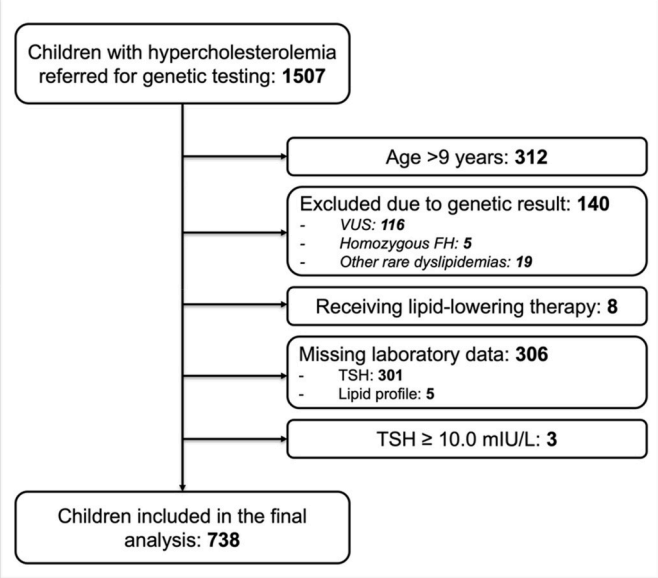



**Fig. 2.** Cohort selection flow diagram.
**Abbreviations: TSH** – thyroid-stimulating hormone; **VUS** – variant of uncertain significance; **FH** – familial hypercholesterolemia.

### 2.1. Universal screening for FH in Slovenia

Slovenia has operated a universal preschool screening program for FH since 1995, now covering >90% of children each year. At the mandatory 5–6-year examination at primary care, TC is measured. Children are referred to the national reference center—UCH-LJ—when TC is ≥ 6.0 mmol/L or when TC is ≥ 5.0 mmol/L in the presence of a positive family history of premature cardiovascular disease. At the tertiary center, confirmatory diagnostics, including genetic testing, are performed, and if FH is verified, cascade testing is offered to parents and siblings [15–18].

### 2.2. Laboratory assessments and genetic analysis

All analyses were conducted in accredited laboratories of the University Medical Centre Ljubljana with standardized protocols. Fasting venous blood was drawn for a lipid profile—TC, HDL-C, LDL-C, triglycerides, Apolipoprotein B (ApoB) and Apolipoprotein (ApoA1). LDL-C was calculated by the Friedewald equation when TG < 4.0 mmol/L and measured directly when triglycerides ≥4.0 mmol/L. Lipid parameters and additional biochemical analytes were determined using enzymatic colorimetric methods on Siemens ADVIA 1800 analyzer (Siemens Healthineers, Germany) in earlier years and on Abbott Alinity C Chemistry Analyzers (Abbott Laboratories, USA) following laboratory modernization. ApoB and ApoA1 were measured by immunonephelometry using the Siemens Atellica Neph 630 system (Siemens Healthineers, Germany). TSH was measured at the same visit as the lipid profile using immunochemical methods as part of routine clinical diagnostics. During the study period, laboratory platforms evolved in accordance with routine modernization. Earlier measurements were performed using the ADVIA Centaur system (Siemens Healthineers, Germany), while in more recent years thyroid function tests have been measured using the Siemens Atellica analyzer (Siemens Healthineers, Germany).

Genetic diagnostics focused on the three principal FH genes (*LDLR*, *APOB*, *PCSK9*). During the study period, next-generation sequencing (targeted panel) was used to detect variants in these genes. Sanger sequencing was applied for confirmation and for cascade testing of siblings of genetically confirmed cases. Variants were interpreted according to the American College of Medical Genetics and Genomics (ACMG) guidelines [19,20]; pathogenic and likely pathogenic variants defined an FH-positive result, while benign/likely benign were considered negative. Individuals with VUS in major FH genes were excluded from analyses.

### 2.3. Statistical methods

Data were assembled in Excel 365 (Microsoft, USA) and analyzed in R 4.3.3 (R Foundation, Austria). For each analysis, cases with missing values were listwise excluded; outliers were retained. Continuous variables were non-normally distributed by Shapiro–Wilk testing and are summarized as median (Q1–Q3). Group comparisons (by sex, FH status, and pathogenic-variant subgroup within FH-positive) used the Wilcoxon rank-sum test for continuous variables and the $\chi^2$ test for categorical variables.

Crude associations between TSH and the lipid profile (TC, LDL-C, HDL-C, triglycerides), as well as ApoA1 and ApoB, were assessed using Spearman rank correlations in the overall cohort and after stratification by sex and by FH status. Within the FH-positive subgroup, correlations were repeated after stratifying by genes with pathogenic variant (*LDLR* vs. *APOB*). Multiple testing was controlled with the Benjamini–Hochberg false-discovery rate (FDR) within each family of hypotheses (e.g., per table/figure).

We assessed whether the TSH–LDL-C relationship differs by FH status using a linear model with an interaction term (LDL-C ~ TSH × FH status), adjusting for age and sex. From this model we estimated the interaction and the TSH slope within each FH group. In FH-positive

children, we repeated the analysis to compare pathogenic variants (*LDLR* vs. *APOB*) using an analogous model (LDL-C ~ TSH × variant + age + sex), reporting variant-specific slopes and the interaction.

All tests were two-sided; statistical significance was defined as $p < 0.050$.

## 3. Results

### 3.1. Study population and baseline characteristics

We included 738 prepubertal children (≤9 years) referred for hypercholesterolemia. Of these, 182 (24.7%) were FH-positive, including 132 (72.5%) with pathogenic *LDLR* variants and 50 (27.5%) with *APOB* variants; no *PCSK9* pathogenic variants were identified. Baseline characteristics are summarized in Table 1. Overall, 45 children (6.1%) had TSH values above the upper reference limit (>4.16 mIU/L) with normal free thyroxine and no clinical features of overt hypothyroidism, consistent with subclinical hypothyroidism.

Lipid profiles and TSH values were similar between boys and girls, with the exception of triglycerides, which were modestly higher in girls than boys (0.9 [0.7–1.2] vs 0.8 [0.6–1.2] mmol/L; $p = 0.014$). Among FH-positive children, TSH did not differ between *LDLR* and *APOB* carriers ($p = 0.987$). However, *LDLR* carriers had higher TC and LDL-C than *APOB* carriers (TC 6.70 [6.00–7.73] vs 6.40 [6.10–6.68] mmol/L, $p = 0.040$; LDL-C 4.80 [4.10–5.65] vs 4.60 [4.20–4.90] mmol/L, $p = 0.043$), while age, HDL-C, triglycerides, ApoB, and ApoA1 were comparable between groups (all $p > 0.050$).

### 3.2. Correlations between TSH and lipid parameters

In the overall cohort, TSH showed no correlation with age or any lipid parameter (all $p \geq 0.050$). A weak unadjusted association with triglycerides (Spearman $\rho = 0.078$; $p = 0.035$) did not remain significant after FDR correction (adjusted $p = 0.061$).

After sex stratification, TSH correlated with triglycerides in males ($\rho = 0.156$; $p = 0.012$) but not in females ($\rho = 0.015$; $p = 0.850$). When stratified by FH status, a different pattern emerged. In FH-positive children, TSH correlated positively with TC, LDL-C, and ApoB ($\rho = 0.184$, 0.191, and 0.207; $p = 0.027$, 0.023, and 0.020, respectively). In FH-negative children, correlations with TC and ApoB were weaker, and no association with LDL-C was observed ($\rho = 0.091$, $p = 0.061$). HDL-C was not associated with TSH in any subgroup.

Within the FH-positive group, gene-stratified analyses showed that TSH correlated with TC and LDL-C among *LDLR* carriers ($\rho = 0.240$ and 0.237; both $p = 0.019$), with a borderline association for ApoB ($\rho = 0.205$; $p = 0.051$). In contrast, no correlations between TSH and TC, LDL-C, or ApoB were observed among *APOB* carriers ($\rho = -0.019$, −0.012, and 0.188; $p = 0.932$, 0.932, and 0.352).

**Table 1**
Baseline characteristics of the study cohort by FH status.

| | Overall | FH-positive | FH-negative | p-value |
|---|---|---|---|---|
| **Female sex [%]** | 436 (59.0%) | 93 (51.1%) | 343 (61.7%) | 0.012 |
| **Age [years]** | 6.3 [5.8–7.1] | 6.39 [5.8–7.4] | 6.25 [5.8–7.0] | 0.167 |
| **TSH [mIU/L]** | 2.30 [1.68–3.11] | 2.20 [1.59–3.14] | 2.33 [1.73–3.1] | 0.196 |
| **TC [mmol/L]** | 5.6 [5.1–6.2] | 6.5 [6.0–7.3] | 5.4 [4.9–5.9] | <0.001 |
| **HDL-C [mmol/L]** | 1.5 [1.3–1.8] | 1.4 [1.2–1.6] | 1.6 [1.4–1.8] | <0.001 |
| **LDL-C [mmol/L]** | 3.6 [3.1–4.2] | 4.8 [4.2–5.4] | 3.3 [2.9–3.7] | <0.001 |
| **Triglycerides [mmol/L]** | 0.8 [0.6–1.2] | 0.8 [0.6–1.1] | 0.8 [0.6–1.2] | 0.569 |
| **ApoA1 [g/L]** [a] | 1.53 [1.37–1.69] | 1.38 [1.26–1.51] | 1.56 [1.44–1.71] | <0.001 |
| **ApoB [g/L]** [b] | 0.99 [0.87–1.13] | 1.23 [1.12–1.39] | 0.94 [0.84–1.04] | <0.001 |

N = 738; FH-positive N = 182, FH-negative N = 556.
Values are median [Q1-Q3] for continuous variables and percentage for categorical variables. p-values compare FH-positive vs FH-negative: $\chi^2$ test for Female sex; Wilcoxon rank-sum test for continuous variables (two-sided).
FH-positive = genetically confirmed monogenic FH; FH-negative = no pathogenic variant (polygenic/negative testing).
**Footnotes:** [a] ApoA1 missing 56 observations; [b] ApoB missing 59 observations.
**Abbreviations**: FH – familial hypercholesterolemia; TSH – thyroid-stimulating hormone; TC – total cholesterol; HDL-C – high-density lipoprotein cholesterol; LDL-C – low-density lipoprotein cholesterol; ApoA1 – apolipoprotein A1; ApoB – apolipoprotein B.

### 3.3. Multivariable analysis: interaction between TSH and FH status

To formally test whether the TSH–LDL-C association differed by FH status, we fitted a linear regression model including an interaction term (LDL-C ~ TSH × FH status + age + sex). Model fit was good ($R^2 = 0.441$; overall $p < 0.001$).

The TSH × FH interaction was statistically significant ($\beta = 0.141$ mmol/L per mIU/L, SE = 0.062, $p = 0.023$), indicating a steeper TSH–LDL-C slope in FH-positive compared with FH-negative children. In FH-negative children, the association between TSH and LDL-C was small and not significant ($\beta = 0.037$ mmol/L per mIU/L; $p = 0.235$). In FH-positive children, the implied slope was $\beta = 0.178$ mmol/L per mIU/L ($p = 0.001$). FH-positive status itself was associated with a markedly higher LDL-C level ($\beta = 1.210$ mmol/L; $p < 0.001$). Age and male sex showed borderline negative associations with LDL-C ($\beta = -0.046$, $p = 0.090$; $\beta = -0.113$, $p = 0.048$, respectively).

These opposing slopes explain the absence of an association in the pooled cohort, where the shallow FH-negative and steeper FH-positive slopes average toward no overall correlation. Adjusted predicted relationships are shown in Fig. 3A.

### 3.4. FH-positive subgroup: gene-specific differences in the TSH–LDL-C association

Among FH-positive children, we further assessed whether the TSH–LDL-C association differed by defective gene using a gene-interaction model adjusted for age and sex (LDL-C ~ TSH × defective gene + age + sex). Model fit was modest ($R^2 = 0.149$; overall $p < 0.001$).

A significant positive association between TSH and LDL-C was observed in *LDLR* carriers ($\beta = 0.237$ mmol/L per mIU/L; $p = 0.004$), whereas no association was present among *APOB* carriers ($\beta = -0.065$ mmol/L per mIU/L; $p = 0.655$). The TSH × defective gene interaction was borderline significant ($\beta = -0.302$; $p = 0.072$), suggesting heterogeneity with a steeper TSH–LDL-C relationship in *LDLR*-mediated FH. Age was inversely associated with LDL-C ($\beta = -0.192$; $p = 0.001$), while sex and the APOB vs LDLR main effect were not significant. Adjusted predicted lines are shown in Fig. 3B.

## 4. Discussion

In this study, we investigated whether circulating TSH relates to the lipid profile in prepubertal children with hypercholesterolemia, and whether any association depends on FH status or the underlying defective gene. In the pooled cohort, TSH did not correlate with age or lipids. When stratified, associations emerged: in FH-positive children, higher TSH aligned with higher LDL-C, driven by carriers of *LDLR* pathogenic variants and not observed in *APOB* carriers; in FH-negative children the TSH–LDL-C link was weak to absent. Given the cross-sectional design, we cannot determine the direction of this association, and the possibility of reverse causality—whereby LDL-C or FH-related metabolic pathways influence TSH—cannot be excluded. While the effect size is modest—around +0.36 mmol/L LDL-C for a 2 mIU/L TSH increase—it could still hold some relevance over time in FH, where cumulative LDL-C exposure matters.

The unique structure of Slovenia's universal preschool FH screening program provides an exceptionally homogeneous and well-

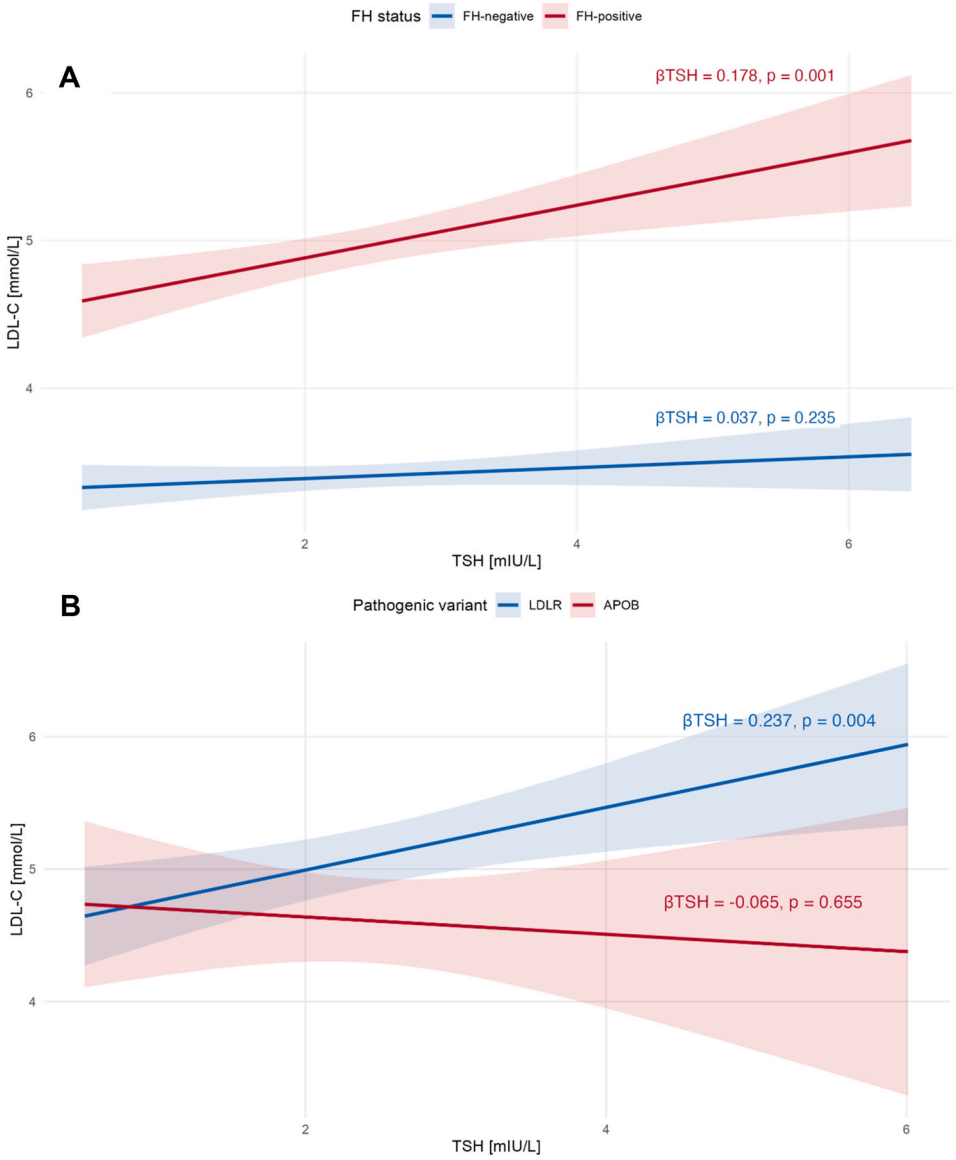



**Fig. 3. Adjusted association of LDL-C with TSH.**

**Abbreviations:** TSH – thyroid-stimulating hormone; LDL-C – low-density lipoprotein cholesterol; FH – familial hypercholesterolemia; *LDLR* – *LDL receptor* gene; APOB – *apolipoprotein B* gene; β – regression slope; CI – confidence interval.

**(A)** Whole cohort. Lines show model-based predictions from a linear model (*LDL-C ~ TSH × FH status + age + sex*), evaluated at the cohort median age (6.31 y) and female sex. **(B)** FH-positive subgroup. Predictions from *LDL-C ~ TSH × defective gene (LDLR vs APOB) + age + sex*, evaluated at median age (6.39 y) and female sex. Shaded areas denote 95% CIs.

characterized pediatric cohort, allowing more precise evaluation of additional physiological and hormonal factors that may influence cholesterol levels [14,15].

Early kinetic studies showed that hypothyroid patients have impaired LDL-C binding/clearance—functionally resembling FH—suggesting that reduced LDLR activity is central to the dyslipidemia of thyroid deficiency [2]. T3 up-regulates *LDLR* via SREBP-2 [7], and also modulates other lipid pathways: it increases hepatic HMG-CoA reductase expression (cholesterol synthesis), potentially affects intestinal cholesterol absorption through NPC1L1, and influences triglyceride handling (e.g., through lipoprotein lipase) and HDL-C remodeling. In overt hypothyroidism, the net effect of down-regulated *LDLR* and increased absorption outweighs reduced synthesis, yielding higher TC, LDL-C and ApoB; many of these abnormalities improve with levothyroxine [21]. Consistent with this framework, adult population studies suggest that TSH itself may exert both thyroid hormone–dependent and –independent effects on lipid metabolism. In particular, Wang et al. demonstrated that higher TSH levels within the reference range were associated with higher TC and triglycerides even after adjustment for free T3 and T4 [8].

Our findings align with this biology: within FH-positive children—particularly those carrying pathogenic *LDLR* variants—TSH tracked with TC, LDL-C and ApoB, whereas no such pattern was evident in *APOB* carriers. This is biologically plausible if even modest thyroid-driven reductions in LDLR expression further constrain an already insufficient receptor pathway, amplifying LDL-C responses in FH caused by *LDLR* pathogenic variants. By contrast, when the primary defect is in *APOB*, variation in receptor abundance may have less phenotypic leverage on LDL-C. Whether the arrow could also point the other way—i.e., *LDLR* defects altering pituitary–thyroid homeostasis—remains speculative. We are not aware of human evidence that heterozygous *LDLR* pathogenic variants per se shift TSH or thyroid hormone set-points, and no established mechanistic loop links *LDLR* to hypothalamic–pituitary–thyroid regulation. This magnified impact of subclinical

hypothyroidism in *LDLR* carriers highlights the central role of the LDLR pathway in determining LDL-C levels in FH, a pathway that is targeted by statin therapy through activation of SREBP-2 and up-regulation of LDLR expression [22]. Statins are well-studied and safe in children with FH and are indicated from about 8 years of age [23,24]. Importantly, the aim of this study was not to assess treatment response but to explore whether thyroid function contributes to early inter-individual variability in LDL-C among children with FH. In this context, our findings suggest that subclinical alterations in thyroid function may act as a modifier of LDL-C expression in LDLR-mediated FH. This information may be relevant for early risk assessment and metabolic phenotyping, rather than immediate therapeutic decision-making.

In our hypercholesterolemic, largely prepubertal cohort without clinically overt hypothyroidism, the absence of a clear TSH–cholesterol association in FH-negative children and in *APOB* carriers likely reflects both design and biology. Range restriction limits signal (FH-negative referrals cluster around elevated LDL-C cut-points, and we excluded overt thyroid dysfunction), and any TSH effect on LDL-C is smaller and more heterogeneous when the LDLR pathway is intact or variably influenced by *APOB*, but amplified in *LDLR*-mediated FH—where we observed the strongest coupling [21]. This helps reconcile our findings with prior pediatric reports that, in broader or hypothyroid-enriched samples, show links between higher TSH and a more atherogenic profile [10–12]. Additional support for such modest thyroid–lipid interactions comes from recent work in paediatric obesity, where subclinical hypothyroidism was associated with higher triglycerides and where longitudinal changes in TSH paralleled changes in LDL-C and TC [25]. Consistent with this, we observed a weak but significant correlation between TSH and triglycerides in boys, but not in girls, suggesting that sex-specific factors may modulate the sensitivity of triglyceride metabolism to thyroid status even in early childhood. A recent study of patients labeled as FH—defined largely by clinical criteria, spanning older ages and mixed pubertal status, and lacking gene-based stratification—did not find a clear association between TSH and cholesterol within the FH-positive subgroup [26]. In contrast, our cohort is genetically confirmed and predominantly prepubertal, which reduces confounding from puberty, sex hormones, adiposity, and treatment; under these cleaner conditions, we observe that the TSH–LDL-C relationship is most apparent in children with *LDLR* pathogenic variants.

Our gene-stratified findings suggest that subclinical thyroid dysfunction may have a disproportionate impact on LDL-C in *LDLR*-FH, potentially reflecting limited receptor reserve. Although our cross-sectional data cannot establish causality, this pattern raises the possibility that even mild elevations in TSH could further challenge LDLR-mediated clearance. Clinically, this signal may justify closer monitoring of thyroid function in *LDLR*-FH and consideration of a lower threshold for treating subclinical hypothyroidism on an individualized basis, particularly when LDL-C remains above target despite optimized statin ± ezetimibe therapy. In adults with *LDLR*-FH, such an approach is biologically plausible and hypothesis-generating, but requires prospective confirmation before firm recommendations can be made. By contrast, the absence of a TSH–LDL-C association in *APOB*-FH suggests that thyroid status may not influence LDL-C uniformly across genotypes, underscoring the need for genotype-informed rather than universal strategies.

This study has several limitations. First, its retrospective, cross-sectional design precludes causal inference and captures thyroid status and lipids at a single time point. Second, we evaluated only children referred for hypercholesterolemia; this range restriction likely attenuates modest TSH–lipid associations—an effect that would probably be magnified in a cohort including normocholesterolemic children. Third, multivariable models were adjusted for age and sex only, systematic measurements of free T3 and T4 were not available for all participants, precluding formal assessment of thyroid hormone–independent effects of TSH. In addition, thyroid peroxidase antibodies and family history of thyroid disease were not systematically collected, as thyroid evaluation in this primary hypercholesterolemia cohort was limited to TSH screening for secondary causes. We did not account for potential confounders such as adiposity (e.g., BMI or BMI-z score), diet, physical activity, insulin resistance, or thyroid autoimmunity. Finally, the single-center setting may limit generalizability beyond our referral population and healthcare context.

## 5. Conclusions

In prepubertal children referred for hypercholesterolemia, TSH showed no overall association with lipids. However, within FH due to *LDLR* variants, higher TSH was associated with higher LDL-C—and with TC and ApoB—while no such pattern appeared in *APOB* carriers or FH-negative children. This gene-specific signal is consistent with LDL-receptor biology and suggests that thyroid status may have differential relevance across FH genotypes. In clinical practice, thyroid function testing is already part of standard baseline assessment in pediatric FH. In selected cases—particularly in children with *LDLR*-mediated FH and suboptimal LDL-C control—thyroid status may represent a modifiable contributor to LDL-C expression, and repeat assessment of thyroid function could be considered as part of a broader metabolic evaluation. These findings are hypothesis-generating, and prospective studies are required before any therapeutic implications can be defined.

## Ethics considerations

The study was approved by the Slovenian National Medical Ethics Committee (0120-14/2017/5; 0120-100/2019/5). The study was conducted in accordance with the Declaration of Helsinki. Informed consent for genetic analysis and publication of anonymized data, was obtained from parents or legal guardians.

## Originality of content

We confirm that all information and materials in the manuscript are original.

## Author contribution-CRediT

Conceptualization: J.S., J.Ko., T.B., U.G.; Methodology: J.Ka., J.S., M.P.J., J.Ko.; Data Curation: J.Ka., J.S., M.M., B.C.K., M.P.J., A.D.T., T. G., A.P., A.L.; Formal Analysis: J.Ka., M.P.J; Investigation: M.M., B.C.K., A.D.T., T.G., A.P., A.L.; Supervision: T.B., J.Ko., U.G.; Funding Acquisition: T.B., J.Ko., U.G.; Writing – Original Draft: J.Ka., J.S., U.G.; Writing – Review & Editing: All authors.

## Financial support

This work was supported by the Slovenian Research and Innovation Agency (grants: P3-0343, J3-2536). The funding organization had no role in the design and conduct of the study; collection, management, analysis, and interpretation of the data; preparation, review, or approval of the manuscript; and decision to submit the manuscript for publication.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Acknowledgments

We sincerely thank all the children and families who participated in this study. U.G. has had full access to all study data and takes responsibility for its integrity and accuracy. We acknowledge the use of

artificial intelligence (AI)-based natural language processing tools for language editing and minor assistance with coding and data processing. These tools were used to improve clarity and readability and to facilitate certain computational tasks. No AI tool was involved in generating original scientific content, analysis, or interpretation. Additionally, we acknowledge the contributions of all clinicians, researchers, and collaborators who assisted in data collection, analysis, and manuscript preparation.

## Data availability

Data are available from the corresponding author upon reasonable request.

## References


[1] Ference BA, Ginsberg HN, Graham I, Ray KK, Packard CJ, Bruckert E, et al. Low-density lipoproteins cause atherosclerotic cardiovascular disease. 1. Evidence from genetic, epidemiologic, and clinical studies. A consensus statement from the European atherosclerosis society consensus panel. Eur Heart J 2017;38(32):2459–72.

[2] Brown MS, Goldstein JL. Familial hypercholesterolemia: defective binding of lipoproteins to cultured fibroblasts associated with impaired regulation of 3-hydroxy-3-methylglutaryl coenzyme A reductase activity. Proc Natl Acad Sci USA 1974;71(3):788–92.

[3] Soutar AK, Naoumova RP. Mechanisms of disease: genetic causes of familial hypercholesterolemia. Nat Clin Pract Cardiovasc Med 2007;4(4):214–25.

[4] Stone NJ. Secondary causes of hyperlipidemia. Med Clin North Am 1994;78(1):117–41.

[5] Yanai H, Yoshida H. Secondary dyslipidemia: its treatments and association with atherosclerosis. Glob Health Med 2021;3(1):15–23.

[6] Thompson GR, Soutar AK, Spengel FA, Jadhav A, Gavigan SJ, Myant NB. Defects of receptor-mediated low density lipoprotein catabolism in homozygous familial hypercholesterolemia and hypothyroidism in vivo. Proc Natl Acad Sci U S A 1981;78(4):2591–5.

[7] Shin DJ, Osborne TF. Thyroid hormone regulation and cholesterol metabolism are connected through sterol regulatory element-binding Protein-2 (SREBP-2). J Biol Chem 2003;278(36):34114–8.

[8] Wang F, Tan Y, Wang C, Zhang X, Zhao Y, Song X, et al. Thyroid-stimulating hormone levels within the reference range are associated with serum lipid profiles independent of thyroid hormones. J Clin Endocrinol Metab 2012;97(8):2724–31.

[9] Cheserek MJ, Wu GR, Ntazinda A, Shi YH, Shen LY, Le GW. Association between thyroid hormones, lipids and oxidative stress markers in subclinical hypothyroidism. J Med Biochem 2015;34(3):323–31.

[10] Vukovic R, Zeljkovic A, Bufan B, Spasojevic-Kalimanovska V, Milenkovic T, Vekic J. Hashimoto thyroiditis and dyslipidemia in childhood: a review. Front Endocrinol 2019;10:868.

[11] Unal E, Akin A, Yildirim R, Demir V, Yildiz I, Haspolat YK. Association of subclinical hypothyroidism with dyslipidemia and increased carotid intima-media thickness in children. J Clin Res Pediatr Endocrinol 2017;9(2):144–9.

[12] Dahl AR, Iqbal AM, Lteif AN, Pittock ST, Tebben PJ, Kumar S. Mild subclinical hypothyroidism is associated with paediatric dyslipidaemia. Clin Endocrinol 2018;89(3):330–5.

[13] Vigone MC, Capalbo D, Weber G, Salerno M. Mild hypothyroidism in childhood: who, when, and how should be treated? J Endocr Soc 2018;2(9):1024–39.

[14] Klancar G, Groselj U, Kovac J, Bratanic N, Bratina N, Trebusak Podkrajsek K, et al. Universal screening for familial hypercholesterolemia in children. J Am Coll Cardiol 2015;66(11):1250–7.

[15] Groselj U, Kovac J, Sustar U, Mlinaric M, Fras Z, Podkrajsek KT, et al. Universal screening for familial hypercholesterolemia in children: the Slovenian model and literature review. Atherosclerosis 2018;277:383–91.

[16] Sustar U, Kordonouri O, Mlinaric M, Kovac J, Arens S, Sedej K, et al. Universal screening for familial hypercholesterolemia in 2 populations. Genet Med 2022;24(10):2103–11.

[17] Kafol J, Miranda B, Sikonja R, Sikonja J, Wiegman A, Medeiros AM, Alves AC, Freiberger T, Hutten BA, Mlinaric M, Battelino T, Collaborators, Humphries SE, Bourbon M, Groselj U. Proposal of a familial hypercholesterolemia pediatric diagnostic score (FH-PeDS). Eur J Prev Cardiol 2025 Jun;20:zwaf352. https://doi.org/10.1093/eurjpc/zwaf352.

[18] Groselj U, Wiegman A, Gidding SS. Screening in children for familial hypercholesterolaemia: start now. Eur Heart J 2022;43(34):3209–12.

[19] Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American college of medical genetics and genomics and the association for molecular pathology. Genet Med 2015;17(5):405–24.

[20] Chora JR, Medeiros AM, Alves AC, Bourbon M. Analysis of publicly available LDLR, APOB, and PCSK9 variants associated with familial hypercholesterolemia: application of ACMG guidelines and implications for familial hypercholesterolemia diagnosis. Genet Med 2018;20(6):591–8.

[21] Pearce EN. Update in lipid alterations in subclinical hypothyroidism. J Clin Endocrinol Metab 2012;97(2):326–33.

[22] Wong J, Quinn CM, Brown AJ. SREBP-2 positively regulates transcription of the cholesterol efflux gene, ABCA1, by generating oxysterol ligands for LXR. Biochem J 2006;400(3):485–91.

[23] Luirink IK, Wiegman A, Kusters DM, Hof MH, Groothoff JW, de Groot E, et al. 20-Year Follow-up of statins in children with familial hypercholesterolemia. N Engl J Med 2019;381(16):1547–56.

[24] Wiegman A, Gidding SS, Watts GF, Chapman MJ, Ginsberg HN, Cuchel M, et al. Familial hypercholesterolaemia in children and adolescents: gaining decades of life by optimizing detection and treatment. Eur Heart J 2015;36(36):2425–37.

[25] Tersander B, Olsson R, Aydin BK, Stenlid R, Ciba I, Manell H. Obesity-related subclinical hypothyroidism in childhood: elevated triglycerides but not basal metabolic rate. Pediatr Res 2025;98(1):182–7.

[26] Heidarpour M, Rezvanian P, Sadri MA, Keshavarzrad P, Zakeri R, Vakilbashi O, et al. The association of thyroid-stimulating hormone (TSH) levels and lipid profile in euthyroid patients with familial hypercholesterolemia. BioMed Res Int 2023;2023:4711275.