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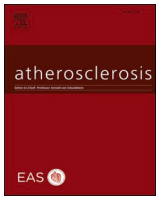

Prevalence, genetic variants, and clinical implications of hypocholesterolemia in children

Urh Groselj [a,b,1], Jan Kafol [a,b,1], Neza Molk [a], Katarina Sedej [c], Matej Mlinaric [a,b], Jaka Sikonja [b,e], Ursa Sustar [a,d], Barbara Cugalj Kern [b,d], Jernej Kovac [b,d], Tadej Battelino [a,b], Marusa Debeljak [b,d,*]

[a] Department of Pediatric Endocrinology, Diabetes and Metabolism, University Children's Hospital, University Medical Centre Ljubljana, Ljubljana, Slovenia
[b] Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia
[c] Community Health Centre Ljubljana, Ljubljana, Slovenia
[d] Clinical Institute of Special Laboratory Diagnostics, University Children's Hospital, University Medical Centre Ljubljana, Ljubljana, Slovenia
[e] Department of Endocrinology, Diabetes and Metabolic Diseases, Division of Internal Medicine, University Medical Centre Ljubljana, Ljubljana, Slovenia

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ABSTRACT

Background and aims: In contrast to extensively studied hypercholesterolemia, knowledge of hypocholesterolemia is limited. This study aims to assess the prevalence, clinical characteristics, and genetics of children and adolescents with hypocholesterolemia.

Methods: This national prospective cross-sectional cohort study was part of Slovenia's universal opt-out cholesterol screening program. The first part assessed hypocholesterolemia prevalence among 3538 children aged 5 years, randomly selected at the mandatory check-up. The second part included analysis of demographic and clinical data and genetic testing of 71 individuals with suspected hypocholesterolemia (total cholesterol [TC] < 3.0 mmol/L [116.0 mg/dL]) referred to the Lipid Clinic of University Children's Hospital Ljubljana.

Results: The prevalence of hypocholesterolemia among 3538 children was 2.66 % (95 % CI: 2.13–3.19 %). Among the 71 genetically tested individuals with suspected hypocholesterolemia, those with pathogenic variants had lower TC (2.58 ± 0.44 mmol/L vs. 2.85 ± 0.42 mmol/L [99.77 ± 17.02 mg/dL vs. 110.20 ± 16.24 mg/dL]; $p = 0.037$) and low-density lipoprotein cholesterol (1.00 ± 0.40 mmol/L vs. 1.33 ± 0.40 mmol/L [38.67 ± 15.47 mg/dL vs. 51.43 ± 15.47 mg/dL]; $p = 0.014$) compared to those without such variants. Genetic testing identified pathogenic alterations in 15 subjects, including 4 novel loss-of-function variants in the APOB gene. All but one subject were asymptomatic.

Conclusions: This study provides new clinical and genetic insights into hypocholesterolemia. Asymptomatic patients with hypocholesterolemia may not require further evaluation, but additional research is needed to understand hypocholesterolemia better.

1. Introduction

Cholesterol is essential for the normal functioning of the human organism, serving various structural as well as regulatory roles [1]. Primary hypocholesterolemia, classified among dyslipidemias, is an inherited disorder of lipid metabolism characterized by total cholesterol (TC) and/or low-density lipoprotein cholesterol (LDL-C) levels below the 5th percentile of the general population, adjusted for age and sex [2]. Several genetic causes of hypocholesterolemia are known, with the most common being familial hypobetalipoproteinemia and abetalipoproteinemia, characterized by alterations in the Apolipoprotein B (*APOB*), Proprotein Convertase Subtilisin/Kexin Type 9 (*PCSK9*), and Microsomal Triglyceride Transfer Protein (*MTTP*) genes [3]. Additionally, hypocholesterolemia is associated with rare conditions stemming from genetic alterations in other genes [4]. Heterozygous carriers of pathogenic variants typically remain asymptomatic, occasionally

* Corresponding author. Clinical Institute of Special Laboratory Diagnostics, University Children's Hospital, University Medical Centre Ljubljana, Bohoriceva 20, 1000, Ljubljana, Slovenia.
E-mail address: marusa.debeljak@kclj.si (M. Debeljak).
[1] These authors contributed equally as first authors.

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exhibiting mild liver dysfunction and hepatic steatosis. Conversely, homozygous individuals with extremely low cholesterol levels may experience severe deficiencies in lipid-soluble vitamins, leading to neurological, hepatic, gastrointestinal, and hematological complications. This highlights the importance of early diagnosis and treatment in such patients [5].

In contrast to hypercholesterolemia, which is extensively studied due to its role as a major causative factor for atherosclerosis and cardiovascular disease-leading causes of global mortality and morbidity- the knowledge of hypocholesterolemia remains limited [6]. This study aims to assess the prevalence, genetic basis, and clinical characteristics of youth with hypocholesterolemia.

2. Patients and methods

2.1. Study design and participants

In this national prospective cross-sectional cohort study, the prevalence of possible hypocholesterolemia (TC < 3.0 mmol/L [116.0 mg/dL]) was calculated in a population-representative sample of 3538 randomly selected preschool children at the age of 5 years who were predominantly of white Caucasian ethnicity, were from all statistical regions in Slovenia and had their lipid measurement performed during mandatory check-ups in the first step of the nation-wide universal cholesterol screening in Slovenia [7,8].

Furthermore, an analysis of the genetic background of hypocholesterolemia was performed in 71 children or adolescents who were referred to the Lipid Clinic at the University Children's Hospital (UCH) Ljubljana, Slovenia, as part of the second step of the screening program (Fig. 1).

In our study, we investigated the clinical impact and genetic background of hypocholesterolemia, primarily detected through universal cholesterol screening that measures TC levels. Consequently, we did not separately assess distinct phenotypes, such as hypobetalipoproteinemia, hypoalphalipoproteinemia, and combined forms, which are associated with hypocholesterolemia [2–4].

We focused on a pediatric population due to the lack of data on how hypocholesterolemia affects children. Additionally, the pediatric population provides an opportunity to better assess genetic influences, given their limited environmental exposure. Early identification of at-risk children allows for timely intervention to prevent potential long-term consequences.

The study adhered to ethical standards as per the Declaration of Helsinki and was approved by the National Medical Ethics Committee of Slovenia (No. 0120-14/2017/5; 0120-100/2019/5; 0120-120/2022/6). All subjects or their legal representatives provided written informed consent for the collection, storage, and analysis of human samples, and were briefed about the conducted research.

2.2. National universal cholesterol screening program

National universal opt-out cholesterol screening, implemented in Slovenia in 1998, includes more than 90 % of individuals from each generation of around 20,000, with hypocholesterolemia also being monitored as part of the screening (Fig. 1). The first step of the screening algorithm consists of TC measurement offered to 5-year-old children as part of mandatory check-ups in each generation at the primary care pediatricians. Children with TC below 3.0 mmol/L (116.0 mg/dL) are optionally referred to the Lipid Clinic at UCH Ljubljana. Considering that referring children with hypocholesterolemia is only a recommendation, not all children are sent to the clinic. In the second step, genetic testing is conducted on referred children with decreased TC levels [7].

2.3. Genetic analysis

Genetic analysis was performed at the UCH Ljubljana, utilizing the whole exome sequencing (WES). Genomic DNA was isolated from peripheral blood according to the established protocols, using the FlexiGene DNA Kit 250 (Qiagen, Hilden, Germany). Libraries were prepared with Illumina DNA Prep with Enrichment kit (Illumina, United States) and xGen Exome Research Panel v2 probes (IDT). The next generation sequencing (NGS) was performed using NovaSeq S4 6000 Reagent Kit

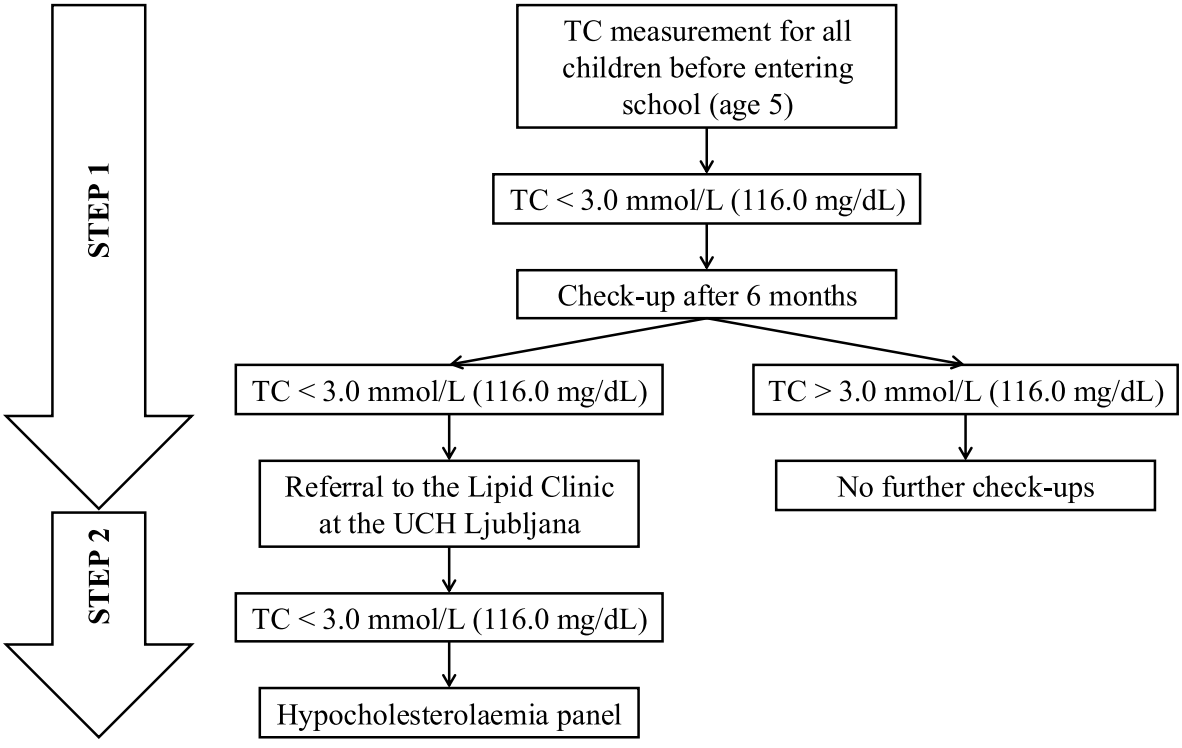

Fig. 1. Hypocholesterolemia screening algorithm in Slovenian pediatric population.

(Illumina, San Diego, CA, United States) according to the manufacturer's instructions and sequenced on the NovaSeq 6000 sequencer (Illumina, San Diego, CA, United States). A panel of genes associated with hypocholesterolemia (Supplemental Table 1), was used for filtering variants. Variant Analysis and Filtration Tool (VarAFT) (Aix Marseille University, France) was utilized for annotation and filtration [9]. The detected genetic variants were classified according to the American College of Medical Genetics and Genomics (ACMG) [10]. For variants of uncertain significance (VUS), likely pathogenic, and pathogenic variants that had sequencing coverage of less than 100 times and/or had a frequency lower than 0.35, validation was conducted using Sanger DNA sequencing (ABI Genetic Analyzer 3500, Applied Biosystems, USA).

2.4. Statistical analysis

Statistical analyses were conducted using R version 4.3.1 (R Foundation for Statistical Computing). Data are presented as mean (± standard deviation or 95 % confidence intervals [CI]) and as frequency (%) for categorical data. Percentiles were calculated based on actual data. Between-group differences were assessed using the Two Sample *t*-test, Mann-Whitney *U* test, and Pearson's Chi-squared test. Two-tailed statistical tests were used and a *p*-value <0.05 was deemed statistically significant.

3. Results

3.1. Prevalence of hypocholesterolaemia

In the Slovenian cohort of 5-years-old children, the proportion of males in the sample was 51.84 % (95 % CI: 50.19%–53.48 %) and the average age was 5.13 years (95 % CI: 4.59–5.67 years). The estimated prevalence of possible hypocholesterolemia was 2.66 % (95 % CI: 2.13–3.19 %). The distribution of all TC values was normal and is presented in Fig. 2. TC at the first percentile was 2.7 mmol/L (104.4 mg/dL), at the third 3.0 mmol/L (116.0 mg/dL), and at the fifth 3.1 mmol/L (119.9 mg/dL).

3.2. Demographic and clinical characteristics

Characteristics of genetically tested subjects are aggregated in Table 1. All of them had TC below 3.0 mmol/L (116.0 mg/dL) at the primary level, and 57 (80.3 %) at the repeat measurement at the UCH Ljubljana, with 19 (26.8 %) having TC below 2.7 mmol/L (104.4 mg/dL). The average age at diagnosis was 7.38 ± 3.05 and 42 subjects (59.2 %) were male. Individuals with a heterozygous pathogenic variant for hypocholesterolemia, in comparison with those without a pathogenic variant, had lower TC (2.58 ± 0.44 mmol/L vs. 2.85 ± 0.42 mmol/L [99.77 ± 17.02 mg/dL vs. 110.20 ± 16.24 mg/dL]; $p = 0.037$) and LDL-C (1.00 ± 0.40 mmol/L vs. 1.33 ± 0.40 mmol/L [38.67 ± 15.47 mg/dL vs. 51.43 ± 15.47 mg/dL]; $p = 0.014$). Liver tests, as well as lipid-soluble vitamins (A, D, E), were all within the normal range, without significant difference between the two groups. Among the subjects with a pathogenic variant, only one was symptomatic, presenting with hepatosplenomegaly and moderate liver fat infiltration, while all other subjects with the variant showed no clinical abnormalities. Among those without a pathogenic variant, two had hepatosplenomegaly, and one additional subject was diagnosed with genetically confirmed Gilbert syndrome.

3.3. Genetic analysis

Fifteen out of 71 subjects (21,1 %) had a (likely) pathogenic variant in genes, associated with hypocholesterolemia (Fig. 3), and were heterozygous and asymptomatic, except for one patient with hepatosplenomegaly, who had a pathogenic variant in APOB (c.6624dup) [11]. Most variants were found in *APOB* (6 variants), followed by *ANGPTL3* (4 variants), *ABCA1* (2 variants), *DHCR7* (2 variants), and *ACOX2* (1 variant). In addition, 4 of those variants in *APOB* (c.908del, c.1265_1276delinsA, c.6624dup, c.9959del; NM_000384) are novel variants, that have previously not been described in the literature or reported in GnomAD and ClinVar databases [11–13].

In 13 (18.3 %) subjects, 14 variants of unknown significance (VUS) were found in genes associated with hypocholesterolemia. 3 VUS in

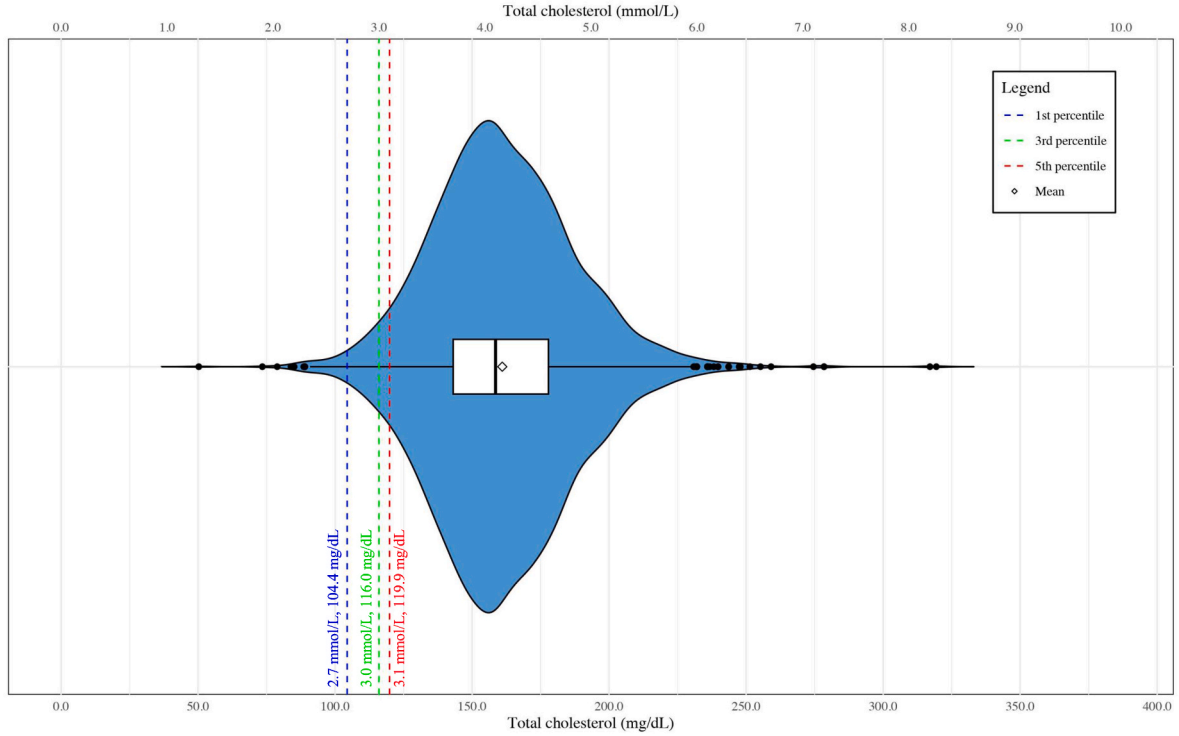

Fig. 2. Distribution of total cholesterol measurements at the first step of the universal screening program for hypercholesterolemia (N = 3538).

Table 1
Demographic and clinical characteristics of subjects, who underwent genetic testing.

	Units	All ± N = 71	Pathogenic variant present[a] ± N = 15	Without pathogenic variant ± N = 56	*p*
Male participants	no. (%)	42 (59)	12 (80)	30 (54)	0.064[c]
Age[b]	years	7.38 ± 3.05	6.47 ± 1.34	7.62 ± 3.33	0.183[d]
Dyslipidemia in family	no. (%)	16 (23)	3 (20)	13 (23)	0.791[c]
TC at screening[b]	mmol/L	2.57 ± 0.29	2.49 ± 0.35	2.59 ± 0.27	0.476[d]
	mg/dL	99.38 ± 11.21	96.29 ± 13.53	100.15 ± 10.44	
TC[b]	mmol/L	2.79 ± 0.44	2.58 ± 0.44	2.85 ± 0.42	**0.037**[e]
	mg/dL	107.89 ± 17.02	99.77 ± 17.02	110.20 ± 16.24	
HDL-C[b]	mmol/L	1.23 ± 0.29	1.24 ± 0.35	1.22 ± 0.27	0.795[e]
	mg/dL	47.56 ± 11.21	47.95 ± 13.53	47.19 ± 10.44	
LDL-C[b]	mmol/L	1.26 ± 0.42	1.00 ± 0.40	1.33 ± 0.40	**0.014**[d]
	mg/dL	48.72 ± 16.24	38.67 ± 15.47	51.43 ± 15.47	
TG[b]	mmol/L	0.83 ± 0.44	0.82 ± 0.61	0.83 ± 0.39	0.497[d]
	mg/dL	73.52 ± 38.97	72.63 ± 54.03	73.52 ± 34.54	
ApoA-I[b]	g/L	1.31 ± 0.22	1.30 ± 0.26	1.32 ± 0.21	0.707[d]
ApoB[b]	g/L	0.45 ± 0.11	0.43 ± 0.13	0.45 ± 0.10	0.536[e]
Total bilirubin[b]	μmol/L	10.03 ± 8.94	10.77 ± 8.15	9.83 ± 9.21	0.770[d]
	mg/dL	0.59 ± 0.52	0.63 ± 0.48	0.57 ± 0.54	
Direct bilirubin[b]	μmol/L	3.80 ± 2.83	4.15 ± 3.34	3.71 ± 2.71	0.869[d]
	mg/dL	0.22 ± 0.17	0.24 ± 0.20	0.22 ± 0.16	
GGT[b]	μkat/L	0.18 ± 0.06	0.19 ± 0.05	0.18 ± 0.06	0.949[d]
	U/L	10.78 ± 3.59	11.38 ± 2.99	10.78 ± 3.59	
AST[b]	μkat/L	0.52 ± 0.14	0.51 ± 0.16	0.52 ± 0.14	0.510[d]
	U/L	31.14 ± 8.38	30.54 ± 9.58	31.14 ± 8.38	
ALT[b]	μkat/L	0.36 ± 0.21	0.47 ± 0.37	0.34 ± 0.14	0.124[d]
	U/L	21.56 ± 12.57	28.14 ± 22.16	20.36 ± 8.38	
Vitamin A[b]	μmol/L	1.28 ± 0.33	1.37 ± 0.40	1.25 ± 0.30	0.254[e]
	μg/dL	36.68 ± 9.46	39.26 ± 11.46	35.82 ± 8.60	
25-hydroxy vitamin D[b]	nmol/L	78.03 ± 30.87	79.50 ± 29.18	77.62 ± 31.60	0.581[d]
	ng/mL	31.26 ± 12.37	31.85 ± 11.69	31.10 ± 11.66	
Vitamin E[b]	μmol/L	19.40 ± 4.26	17.47 ± 4.25	19.97 ± 4.14	0.053[e]
	μg/mL	8.35 ± 1.8	7.52 ± 1.83	8.60 ± 1.78	

ApoA-I = Apolipoprotein A-I; ApoB = Apolipoprotein B; ALT = Alanine aminotransferase; AST = Aspartate aminotransferase; GGT = Gamma-glutamyl transpeptidase; HDL-C = High-density lipoprotein cholesterol; LDL-C = Low-density lipoprotein cholesterol; no. = number; TC = Total cholesterol; TG = Triglycerides.
[a] We included subjects with confirmed pathogenic or likely pathogenic variant.
[b] The result is reported with the standard deviation given in brackets.
[c] Pearson's Chi-squared test.
[d] Mann-Whitney *U* test.
[e] Two Sample *t*-test.

PCSK9 (c.73_81del; NM_174936), *DOCK7* (c.2976G > C; NM_001367561), and *FDFT1* (c.192G > C; NM_001287750) were first detected in this study. VUS and (likely) pathogenic variants are summarized in Supplemental Table 2 and interpreted in Supplemental Material.

4. Discussion

The knowledge of the prevalence of hypocholesterolemia is limited, especially in the pediatric population, and it varies with used cut-off value. Its prevalence among white and black men, aged 26–46 years, was estimated at 1.8 % and 3.6 %, respectively, when using a cut-off value of 3.4 mmol/l (130.0 mg/dL) [14]. Based on cut-off value of 3.0 mmol/L (116.0 mg/dL), the prevalence in Slovenian children was in the same range (2.66 %). Following the classification of hypocholesterolemia as TC under the 5th percentile, the cut-off value should be set at 3.1 mmol/L (119.9 mg/dL) [2].

Despite the random selection of genetically tested subjects, the findings of this study, which provide insights into the distribution of gene variants associated with hypocholesterolemia, should be generalized to the entire Slovenian pediatric population with caution due to the relatively small sample size. Lower levels of TC and LDL-C in subjects with pathogenic variants in heterozygous form suggest that those variants may have a more substantial impact on cholesterol levels than any other factors in investigated study group. Consequently, slight lowering of the referral cut-off value could be considered for the Lipid Clinic to reduce negative genetic analyses.

Blanco-Vaca et al. described an even higher proportion (52.3 % vs. 21.1 % in this study) of pathogenic variants in their analysis of subjects with suspected primary hypocholesterolemia. However, the higher proportion compared to this study may be attributed to more stringent exclusion [15].

The absence of causative genetic alteration in 78.9 % of subjects could potentially be explained by the polygenic origin of hypocholesterolemia [15]. It is also possible that alterations in other genes not covered by the panel, used at UCH Ljubljana, or not yet associated with hypocholesterolemia were responsible. Additionally, secondary hypocholesterolemia should be considered, although it seems unlikely given the unremarkable clinical and laboratory findings [16].

The asymptomatic presentation in most subjects with pathogenic variants was expected, as they were all heterozygous. A heterozygous genotype causes lower cholesterol levels, which do not reach critical values, resulting in significant impairment of growth and development [17]. The lower cholesterol levels likely reduce the risk of developing atherosclerotic cardiovascular disease [5].

Normal levels of lipid-soluble vitamins indicate that substitution might not be required in asymptomatic heterozygous individuals with hypocholesterolemia, whereas in homozygous individuals, such treatment is critical for preventing complications [17]. Based on these findings, asymptomatic patients with mild hypocholesterolemia detected during a screening program may not require referral to a tertiary center for further diagnostics or regular follow-up. However, it is important to note that APOB pathogenic variants have been associated with increased ALT levels and steatosis, as well as the severity of steatosis. The potential relationship between APOB pathogenic variants and hepatic inflammation or fibrosis remains unclear. Therefore, additional data and longer follow-up are necessary to better understand these associations [18].

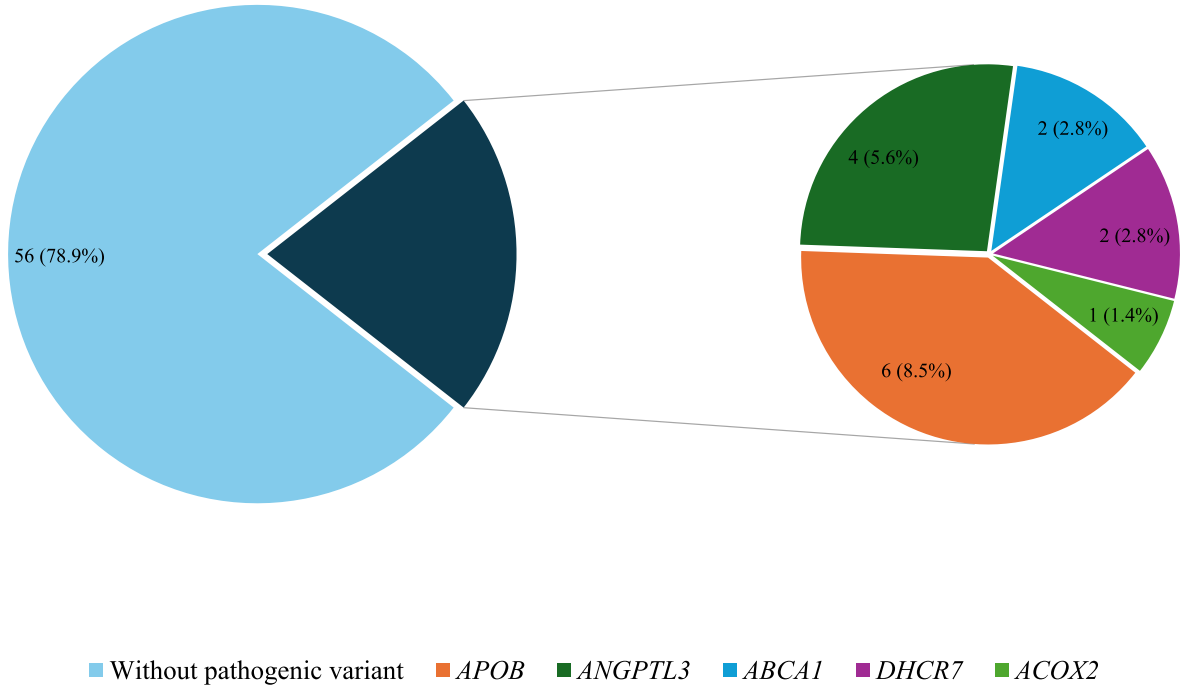

Fig. 3. Proportion of patients with a confirmed (likely) pathogenic variant in a specific gene associated with hypocholesterolemia and proportion of patients without pathogenic variants in those genes (N = 71).

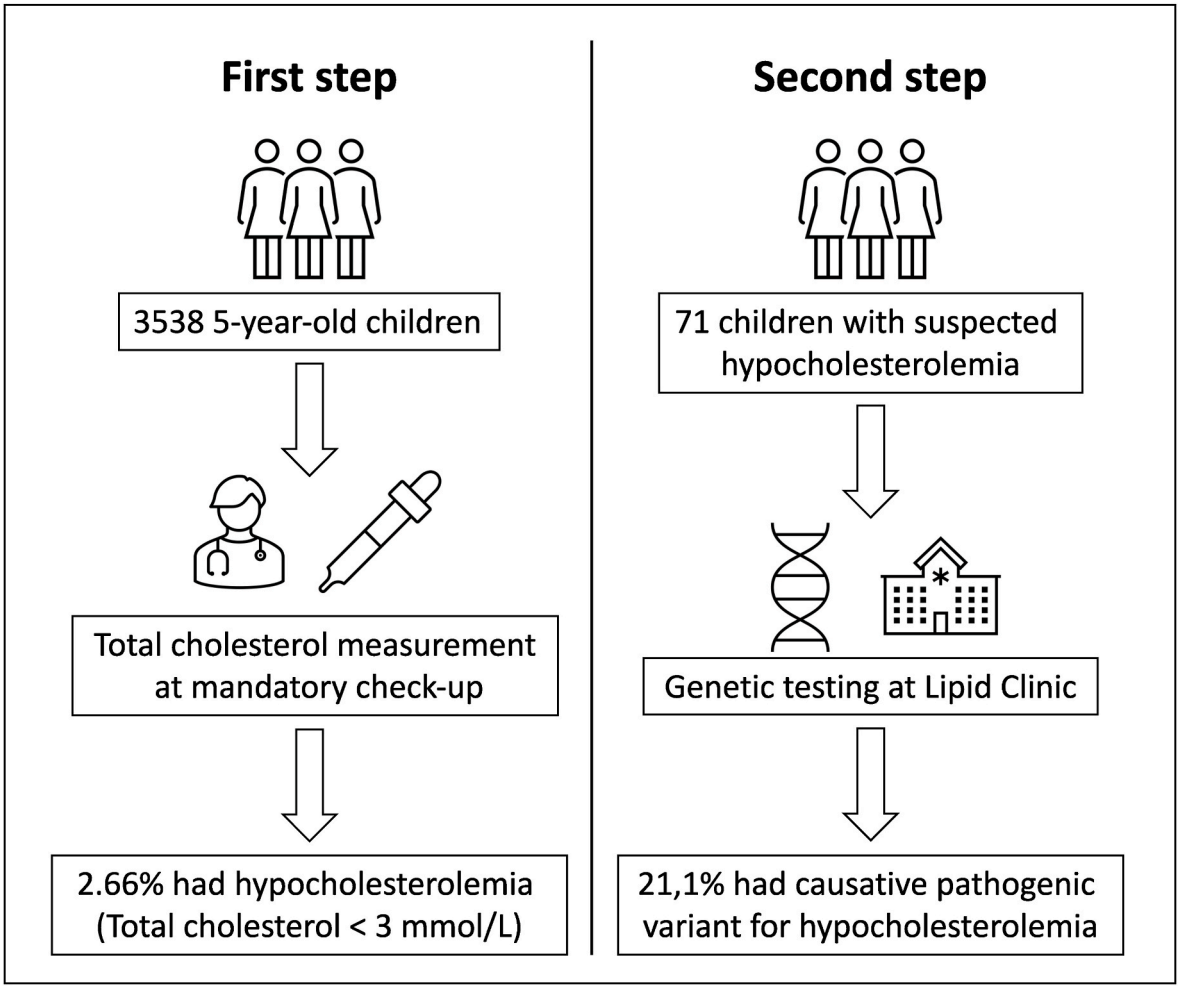

Fig. 4. Graphical abstract.

The study has several limitations. The small sample size of genetically tested subjects and the predominantly white Caucasian cohort may limit the generalizability of findings. The gene panel used might have missed other relevant genetic variants. Cross-sectional data does not allow for long-term outcome assessment or the impact of environmental factors on cholesterol levels. Additionally, some VUS could not be definitively classified. Reevaluating results of this research when new potentially causal genes emerge in future studies to potentially enhance the diversity of genetic variants, could offer a deeper understanding of hypocholesterolemia. The lack of data on the prevalence of a polygenic burden for hypocholesterolemia represents another limitation, as this information could offer important insights into the genetic landscape of the condition. Larger diverse cohorts and longitudinal studies are needed to validate reported results and explore the clinical implications further.

4.1. Conclusion

The prevalence of hypocholesterolemia in children was 2.66 %. Genetic testing identified causative alterations in heterozygous form in 21.1 % of subjects, including 4 novel variants in *APOB*. Subjects with confirmed pathogenic variants were mostly asymptomatic (Fig. 4). Therefore, additional diagnostics may not be needed for asymptomatic children with mild hypocholesterolemia.

CRediT author statement

UG, Investigation: Conceptualization: Formal analysis: Writing – original draft: Writing – review & editing: Validation: JKa: Investigation: Conceptualization: Formal analysis: Writing – original draft: Writing – review & editing: Validation: NM, Investigation: Conceptualization: Formal analysis: Writing – review & editing: Validation: KS: Writing – review & editing: Validation: MM: Writing – review & editing: Validation: JS, Writing – original draft: Writing – review & editing: Validation: US: Writing – review & editing: Validation: BCK: Writing – review & editing: Validation: JK: Writing – review & editing: Validation: TB: Writing – review & editing: Validation: MD: Investigation: Conceptualization: Writing – review & editing: Validation

Data availability statement

Data is available upon reasonable request.

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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.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.atherosclerosis.2024.119065.

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