



Article

Evaluation of Classical and New Clinical Criteria for Diagnosing Familial Hypercholesterolemia in Childhood

Raffaele Buganza^{1,*} , Giulia Massini¹ , Cecilia Nobili¹ , Martina Ferrandino^{2,3} , Maria Donata Di Taranto^{2,3} , Luisa de Sanctis¹ and Ornella Guardamagna¹

¹ Department of Public Health and Pediatric Sciences, Regina Margherita Children's Hospital, University of Turin, 10126 Turin, Italy; giulia.massini@unito.it (G.M.); cecilia.nobili@unito.it (C.N.); luisa.desantis@unito.it (L.d.S.); ornella.guardamagna@unito.it (O.G.)

² Dipartimento di Medicina Molecolare e Biotecnologie Mediche, Università degli Studi di Napoli Federico II, 80145 Naples, Italy; martina.ferrandino@unina.it (M.F.); mariadonata.ditaranto@unina.it (M.D.D.T.)

³ CEINGE-Biotecnologie Avanzate Franco Salvatore, 80145 Naples, Italy

* Correspondence: raffaele.buganza@unito.it

Abstract

Background: Heterozygous familial hypercholesterolemia (HeFH) is characterized by elevated low-density lipoprotein cholesterol (LDL-C) and increased cardiovascular risk, making early diagnosis essential; however, the diagnostic performance of pediatric criteria is heterogeneous. This study evaluated the effectiveness of different diagnostic criteria and scoring systems to select children for genetic testing. **Materials and methods:** A total of 214 pediatric subjects with suspected HeFH were included, recruited from patients followed at a tertiary care center, based on LDL-C levels $\geq$ 95th age- and sex-specific percentile in both the proband and one biological parent. All subjects underwent genetic analysis of the main FH-associated genes (*LDLR*, *APOB*, *PCSK9*). The following diagnostic criteria and scoring systems were retrospectively evaluated and compared with genetic findings: Simon Broome Register (SBR), Dutch Lipid Clinic Network (DLCN), European Atherosclerosis Society (EAS), American Heart Association (AHA), Familial Hypercholesterolemia Canada Network (FH-CAN), Japanese Atherosclerosis Society (JAS), Lipid TransPort Disorders Italian Genetic Network for Italian pediatric patients (LIPIGEN-FH-PED), and the Familial Hypercholesterolemia Pediatric Diagnostic Score (FH-PeDS). **Results:** Pathogenic variants were identified in 91.8% of subjects. Approaches using lower LDL-C thresholds minimized the loss of variant-positive individuals (particularly JAS and FH-PeDS, with a missed diagnoses rate of 1.6%), whereas more restrictive definitions excluded a substantial proportion of affected patients (10.5% SBR, 56.3% DLCN, 6.3% EAS, 6.3% AHA, 7.4% FH-CAN, and 6.3% LIPIGEN-FH-PED). The mutation detection rate (MDR) was $>91\%$ for all examined criteria. **Conclusions:** Several current diagnostic criteria may underestimate the true number of children carrying FH-associated variants. Less selective criteria enable the identification of a greater number of FH-positive individuals while maintaining a high MDR, thus supporting the prioritization of identifying as many affected children as possible in the pediatric setting. This cohort reflects a tertiary referral population rather than the general population; therefore, further studies are needed to evaluate the applicability of our findings to broader public health contexts and screening settings.



Academic Editor: Saskia Nanette Van Der Crabben

Received: 27 February 2026

Revised: 4 May 2026

Accepted: 25 May 2026

Published: 3 June 2026

Copyright: © 2026 by the authors.

Licensee MDPI, Basel, Switzerland.

This article is an open access article distributed under the terms and

conditions of the [Creative Commons](#)

[Attribution \(CC BY\)](#) license.

Keywords: familial hypercholesterolemia; children; diagnostic criteria; LDL-C

1. Introduction

Heterozygous familial hypercholesterolemia (HeFH) is one of the most common genetic diseases, with an estimated prevalence in the general population of 1 in 200–300 individuals [1–3], while the more severe homozygous familial hypercholesterolemia (HoFH) is considerably rarer, with an estimated prevalence in Europe of 1 in 160,000–300,000 [1].

Individuals with HeFH experience persistently elevated low-density lipoprotein cholesterol (LDL-C) levels from birth, and a well-established, dose-dependent, log-linear relationship between cumulative LDL-C exposure and atherosclerotic cardiovascular disease (ASCVD) has been demonstrated [4]. Moreover, the atherosclerotic process is known to begin during childhood [5], making early diagnosis in pediatric age essential for the timely initiation of treatment.

Despite recommendations, HeFH remains underdiagnosed [6], thereby reducing opportunities for timely and effective therapeutic intervention.

Several diagnostic criteria and scoring systems have been developed to support the diagnosis of HeFH and to guide patient selection for genetic testing, which represents the gold standard for diagnosis by evaluating the main genes associated with FH [1,7]: *LDLR* (accounting for more than 90% of FH cases), *APOB* (approximately 5% of cases), and *PCSK9* (less than 1% of cases), all characterized by dominant transmission. The autosomal recessive form is much rarer (less than 1% of FH cases) and results from pathogenic variants in the *LDLRAP1* gene [8].

In adults, the most widely used diagnostic tools for decades have been the Simon Broome Register (SBR) criteria [9] and the Dutch Lipid Clinic Network (DLCN) score [10]. Both rely on pretreatment LDL-C concentrations, clinical manifestations, patterns of dominant inheritance, and the presence of coronary disease in the family. The SBR criteria include different cut-offs for individuals under 16 years of age, whereas the DLCN score does not specify pediatric ranges. The “Make Early Diagnosis to Prevent Early Deaths” (MEDPED) criteria consider total cholesterol (TC) levels and family history; however, they require a prior diagnosis of FH in a relative, which is often unavailable in clinical practice [11].

Several other criteria define specific pediatric thresholds, including those proposed by the European Atherosclerosis Society (EAS) Consensus Panel for children and adolescents in 2015 [1], the American Heart Association (AHA) in 2015 [12], the Familial Hypercholesterolemia Canada network (FH-CAN) in 2018 [13], the Japanese Atherosclerosis Society (JAS) in 2022 [14], and the International Atherosclerosis Society (IAS) in 2023 [15]. These criteria establish different LDL-C cut-offs for the general pediatric population, for subjects with a suggestive family history, and for children with a parent carrying a genetic diagnosis, except for the AHA criteria, which define a single cut-off for all pediatric subjects in the presence of a specific family history. The JAS criteria include both diagnostic thresholds and an age-specific scoring system for children [14]. The LIPIGEN-FH-PED score, proposed by the Lipid TransPort Disorders Italian Genetic Network (LIPIGEN) of the Italian Society for the Study of Atherosclerosis (SISA) [16], is a modified version of the DLCN score with lower LDL-C cut-offs tailored for pediatric patients. More recently, in 2025, the Slovenian group led by Tapol et al., within the framework of a universal pediatric screening program, proposed a new point-based Familial Hypercholesterolemia Pediatric Diagnostic Score (FH-PeDS) [17].

In the present study, we evaluated the application of the different available diagnostic criteria and scoring systems in a pediatric population with hypercholesterolemia and dominant transmission, expanding both the study cohort and the number of criteria analyzed, including more recently proposed ones, compared with a previous study conducted at our

center [18]. At present, this study appears to be the one with the largest number of different criteria analyzed simultaneously in the pediatric population with suspected HeFH.

2. Materials and Methods

2.1. Study Design

This was a retrospective observational study. All data were collected during routine clinical care at our tertiary dyslipidemia center and subsequently analyzed retrospectively for the purposes of this study.

This study aimed to further investigate the application of various criteria and scores proposed by different authors and scientific societies in a pediatric population with suspected HeFH.

To assess the application of diagnostic criteria and scores currently used in clinical practice, the following criteria and scores were retrospectively applied:

- Simon Broome Register (SBR) criteria [9];
- Dutch Lipid Clinic Network (DLCN) score [10];
- European Atherosclerosis Society (EAS) criteria [1];
- American Heart Association (AHA) criteria [12];
- Familial Hypercholesterolemia Canada network (FH-CAN) criteria [13];
- Japanese Atherosclerosis Society (JAS) criteria and score [14];
- Lipid TransPort Disorders Italian Genetic Network (LIPIGEN)-modified version of the DLCN score for Italian pediatric patients (LIPIGEN-FH-PED) [16];
- Familial Hypercholesterolemia Pediatric Diagnostic Score (FH-PeDS) [17].

MEDPED was not applied, as this was not a cascade screening setting, and the International Atherosclerosis Society (IAS) criteria were not applied because they include “LDL-C exceeding a country-specific threshold and a parental history of elevated LDL-C,” which substantially overlap with our inclusion criteria. The term “not applicable” was used for subjects lacking sufficient characteristics for categorization into the defined classes.

All clinical, anamnestic, biochemical and genetic data were extracted from clinical visit reports stored in the hospital’s information system. Diagnostic criteria and scoring systems were then applied based on these records, and subjects were classified into the corresponding categories. Only individuals with complete data for the application of all diagnostic criteria were included, thus ensuring the absence of missing data.

The patients included in the study were hypercholesterolemic children referred by pediatricians, general practitioners or other specialists at our tertiary center to ascertain the etiology. Consequently, all subjects showed elevated LDL-C levels, above the laboratory reference range, defined as ≥ 95 th percentile. For the parents, we applied the same approach to define elevated LDL-C, adjusting for age-specific percentiles due to the wide age range of the parental cohort. For these reasons, the diagnostic performance reported here may not be directly generalizable to population-based screening settings, where LDL-C levels are typically lower and family history information is often incomplete or unavailable.

2.2. Participants

The study included 214 pediatric subjects (aged < 18 years) evaluated between 2004 and 2025 at the Lipid Clinic of the Regina Margherita Children’s Hospital of Turin (Italy), who underwent molecular analysis because they presented with a clinical suspicion of HeFH according to the following inclusion criteria, after 6 months of dietary intervention:

- LDL-C ≥ 95 th age- and sex-related percentile [19];
- Autosomal dominant inheritance pattern of hypercholesterolemia, with at least one biological parent having baseline LDL-C ≥ 95 th age- and sex-related percentile (pre-treatment values were considered for individuals receiving lipid-lowering therapy) [20].

The percentiles for pediatric age are those reported by the Committee on Nutrition of the American Academy of Pediatrics (adapted from the Lipid Research Clinic Pediatric Prevalence Study), with 95th percentiles of LDL-C ranging from 129 to 140 mg/dL, depending on age and sex [19].

The dietary intervention was based on a Mediterranean-style diet and was implemented by an expert nutritionist during the first outpatient visit.

Secondary hypercholesterolemia was excluded based on clinical evaluation and a panel of biochemical tests aimed at ruling out renal and liver disease, hypothyroidism, and diabetes. The tests included creatinine, aspartate aminotransferase (AST), alanine aminotransferase (ALT), thyroid-stimulating hormone (TSH), free thyroxine (FT4), fasting plasma glucose, and glycated hemoglobin (HbA1c).

2.3. Clinical Evaluation and Family History

We obtained detailed medical histories from all patients, systematically recording the presence of dyslipidemia and cardiovascular disease (CVD) in as many family members as possible, and collecting parental lipid profiles when not previously available. The personal medical history also included the assessment of conditions potentially contributing to secondary dyslipidemia, current medications, dietary habits, and physical activity.

A thorough physical examination was then conducted, including the assessment of characteristic clinical signs such as xanthelasma, xanthomas, and corneal arcus, along with anthropometric measurements. Body weight, height, and body mass index (BMI) were compared with World Health Organization (WHO) reference standards [21].

2.4. Lipid Measurements

Blood samples for lipid profiles (TC, high-density lipoprotein cholesterol -HDL-C-, and triglycerides -TG-) were obtained after an overnight fast. Plasma LDL-C was calculated using the Friedewald formula: $LDL-C = TC - [HDL-C + (TG/5)]$. Non-HDL cholesterol was calculated by subtracting HDL-C from TC.

LDL-C values for patients and their parents were compared to age- and sex-specific population percentiles for children [19] and adults [20].

2.5. Molecular Analysis

Before 2019, genetic screening was performed using a stepwise protocol based on traditional methods, as previously described [22,23]. Initially, the *LDLR* gene was analyzed by amplification and direct sequencing of all exons and adjacent intronic regions. If no pathogenic variants were detected, Multiplex Ligation-dependent Probe Amplification (MLPA) was performed to identify large rearrangements in the *LDLR* gene. When *LDLR* variants were absent, sequencing of all exons and flanking intronic regions of the *PCSK9* gene was conducted. If this was also negative, *APOB* gene analysis was performed, including the LDLR-binding region (a portion of exon 26, exon 29, and flanking exon–intron junctions).

After 2019, NGS-based genetic screening was performed using the Devyser FH v2 kit for sequence enrichment and the Illumina MiSeq platform with paired-end reads (2×150 bp). This approach enabled the detection of small variants in *LDLR*, *APOB*, *PCSK9*, *LDLRAP1*, and *APOE* genes, as well as copy number variants in *LDLR* [18]. Sequence analysis was conducted using Amplicon Suite software version 3.5.1 (SmartSeq). Rare variants were assessed for pathogenicity according to FH-specific recommendations of the American College of Medical Genetics and Genomics (ACMG) guidelines [24,25].

According to these guidelines, some variants may be classified as variants of uncertain significance (VUS) due to conflicting evidence regarding their pathogenicity or insufficient evidence to support classification as either pathogenic or benign.

Variant data were compiled using the Genome Aggregation Database (GnomAD) to determine minor allele frequency (MAF) and by consulting pathogenic variant databases such as HGMD Professional, Leiden Open Variation Database (LOVD 3.0), and ClinVar to identify previously reported FH-associated variants [7].

2.6. Statistical Analysis

Categorical data were reported as absolute numbers. Due to non-normal distribution of variables, group comparisons were performed using the Mann–Whitney test for independent samples. Because the variables were non-normally distributed, data are presented as medians with interquartile ranges (Q1–Q3).

Sensitivity, specificity, and mutation detection rate (MDR) were calculated for each clinical diagnostic criterion and score and compared with molecular genetic results. Sensitivity was defined as the ability to identify individuals with variants in the analyzed genes, specificity as the proportion of patients without a variant who did not fulfill the clinical FH criterion, and MDR as the proportion of patients fulfilling the criterion in whom a variant was identified. These metrics are widely accepted for assessing the performance of diagnostic tests against the gold standard, which in this case was the genetic result. These measures allow a clear, quantitative comparison of how accurately each clinical criterion predicts the genetic diagnosis. Exact 95% confidence intervals for sensitivity and specificity were calculated using the Clopper–Pearson method. As our study is primarily descriptive, we assessed the adequacy of our sample size based on the precision of these confidence intervals rather than on a traditional power analysis.

In addition, the univariate logistic regression was performed to assess the association between LDL-C levels and the genetic status, with the area under the receiver operating characteristic curve (AUC) reported as a measure of discriminatory ability. Multivariate logistic regression was not used as the primary analysis because the small number of V– subjects ($n = 17$) resulted in an events-per-variable ratio below the recommended minimum of 10, increasing the risk of unstable coefficient estimates. Statistical analyses were performed with IBM SPSS Statistics, Version 30.0 (IBM Corp, Armonk, NY, USA). A p -value < 0.05 was considered statistically significant.

3. Results

A total of 214 patients, aged 10.5 (7.6–13.3), underwent molecular analysis according to the predefined inclusion criteria.

The present cohort characteristics are summarized in Table 1 and more clinical and genetic details were previously reported [26].

Among the 214 subjects, 190 carried a pathogenic variant (V+), 17 tested negative (V–), and 7 presented a VUS. Among the 190 V+ cases, 187 carried variants in the *LDLR* gene and 3 in the *APOB* gene.

Since VUS cannot be definitively classified as either benign or pathogenic, but may be reclassified as new evidence becomes available, seven patients carrying VUS were excluded from primary analyses. This approach ensured that results were based on clearly defined genetic categories, thereby minimizing potential confounding effects.

To evaluate the robustness of our findings, we conducted sensitivity analyses under alternative assumptions, classifying VUS as either variant-positive (V+) or variant-negative (V–). The seven individuals with VUS exhibited LDL-C levels comparable to V– subjects ($p = 0.799$) and lower than those observed in V+ subjects ($p = 0.079$). Importantly, the sensitivity analyses confirmed that primary results remained consistent regardless of VUS classification.

Table 1. Characteristics of index patients.

	Overall	Pathogenic Variant (V+)	No Variant (V−)	<i>p</i> -Value
Number	207	190	17	
Age (years)	10.5 (7.6–13.3)	10.4 (7.2–13.3)	11.5 (9.7–15.8)	0.186
Sex (male/female)	105/102	97/93	8/9	0.752
Height (cm)	145.0 (127.0–158.0)	143.5 (127.0–157.8)	147.0 (137.7–152.8)	0.738
Height (SDS)	0.2 (−0.6–0.8)	0.2 (−0.7–0.8)	0.3 (−0.0–0.8)	0.646
Weight (kg)	38.8 (27.1–52.6)	38.5 (26.6–52.7)	41.0 (36.0–49.6)	0.377
Weight (SDS)	0.4 (−0.3–1.3)	0.4 (−0.3–1.3)	1.2 (0.3–1.7)	0.119
BMI (kg/m ²)	18.6 (16.0–21.1)	18.6 (16.0–21.1)	19.1 (17.9–21.5)	0.244
BMI (SDS)	0.4 (−0.4–1.3)	0.4 (−0.4–1.2)	1.2 (−0.2–1.8)	0.159
TC (mg/dL)	285 (257–314)	286 (257–315)	275 (251–296)	0.176
LDL-C (mg/dL)	211 (189–245)	212 (191–246)	188 (176–224)	0.038 *
HDL-C (mg/dL)	55 (48–64)	55 (48–64)	60 (47–77)	0.252
TG (mg/dL)	72 (57–91)	72 (56–90)	81 (60–112)	0.345
Non-HDL-C (mg/dL)	225 (203–261)	226 (203–261)	214 (192–243)	0.074

* $p < 0.05$. Adapted from: Buganza et al. “Diagnosis of Familial Hypercholesterolemia in Children: From Clinical Features Through Gene Variants to Polygenic Score” [26].

The only laboratory parameter showing a statistically significant difference between V+ and V− was LDL-C, which was slightly higher in V+ (212 vs. 188 mg/dL, $p = 0.038$).

In a univariate logistic regression, LDL-C was significantly associated with variant-positive status (OR = 1.016 per mg/dL, 95% CI: 1.002–1.030, $p = 0.027$; AUC = 0.65, 95% CI: 0.52–0.78).

As described in our previous study on this population [26], family history of premature cardiovascular disease (pCHD) in first- or second-degree relatives was observed in 49.4% of V+ and 52.9% of V− individuals. Similarly, pCHD in parents was noted in 15.7% and 17.6%, respectively, with no statistically significant differences between groups.

The applied inclusion criteria resulted in an MDR of 91.8%.

The outcomes derived from the application and comparison of the different classical diagnostic criteria and scores are summarized in Tables 2 and 3, organized according to the respective subcategories of each criterion.

Table 2. Patients categorized according to different criteria.

Clinical Criteria		Total	V+	V−
Simon Broome Register (SBR)	Non applicable	22	20	2
	Possible	178	163	15
	Definite	7	7	0
Dutch Lipid Clinic Network (DLCN)	Unlikely	34	26	8
	Possible	90	81	9
	Probable	72	72	0
	Definite	11	11	0
European Atherosclerosis Society (EAS)	Non applicable	14	12	2
	Highly probable	193	178	15
American Heart Association (AHA)	Non applicable	14	12	2
	Clinical diagnosis of HeFH	193	178	15
Familial Hypercholesterolemia Canada network (FH-CAN)	Non applicable	14	12	2
	Severe hypercholesterolemia	3	2	1
	Probable	184	170	14
	Definite	6	6	0

Table 2. Cont.

Clinical Criteria		Total	V+	V−
Japanese Atherosclerosis Society (JAS)—criteria	Non applicable	5	3	2
	Probable	40	34	6
	Definite	162	153	9
Japanese Atherosclerosis Society (JAS)—score	Unlikely	0	0	0
	Possible	5	3	2
	Probable	40	34	6
	Definite	162	153	9
LIPIGEN-modified version of the DLCN score for Italian pediatric patients (LIPIGEN-FH-PED)	Unlikely	0	0	0
	Possible	16	12	4
	Probable	116	105	11
	Definite	75	73	2
Familial Hypercholesterolemia Pediatric Diagnostic Score (FH-PeDS)	Unlikely	5	3	2
	Possible	1	1	0
	Probable	201	186	15
Inclusion criteria		207	190	17

V+: pathogenic variant; V−: no variant; HeFH: heterozygous familial hypercholesterolemia; LIPIGEN: Lipid TransPort Disorders Italian Genetic Network.

Table 3. The ability of the criteria to predict the results of the molecular analysis.

Clinical Criteria		Sensitivity (%)	95% CI	Specificity (%)	95% CI	MDR
Simon Broome Register (SBR)	Possible or Definite	89.5	84.2–93.5%	11.8	1.5–36.4%	91.9
	Definite	3.7	1.5–7.4%	100	80.5–100.0%	100
Dutch Lipid Clinic Network (DLCN)	Possible or Probable or Definite	86.3	80.6–90.9%	47.1	23.0–72.2%	94.8
	Probable or Definite	43.7	36.5–51.1%	100	80.5–100.0%	100
	Definite	5.8	2.9–10.1%	100	80.5–100.0%	100
European Atherosclerosis Society (EAS)	Highly probable	93.7	89.2–96.7%	11.8	1.5–36.4%	92.2
American Heart Association (AHA)	Clinical diagnosis of HeFH	93.7	89.2–96.7%	11.8	1.5–36.4%	92.2
Familial Hypercholesterolemia Canada network (FH-CAN)	Probable or Definite	92.6	87.9–95.9%	17.6	3.8–43.4%	92.6
	Definite	3.2	1.2–6.7%	100	80.5–100.0%	100
Japanese Atherosclerosis Society (JAS)– criteria	Probable or Definite	98.4	95.5–99.7%	11.8	1.5–36.4%	92.6
	Definite	80.5	74.2–85.9%	47.1	23.0–72.7%	94.4
Japanese Atherosclerosis Society (JAS)– score	Possible or Probable or Definite	100	98.1–100%	0	0.0–19.5%	91.8
	Probable or Definite	98.4	95.5–99.7%	11.8	1.5–36.4%	92.6
	Definite	80.5	74.2–85.9%	47.1	23.0–72.7%	94.4
LIPIGEN-modified version of the DLCN score for Italian pediatric patients (LIPIGEN-FH-PED)	Possible or Probable or Definite	100	98.1–100.0%	0	0.0–19.5%	91.8
	Probable or Definite	93.7	89.2–96.7%	23.5	6.8–49.9%	93.2
	Definite	38.4	31.5–45.7%	88.2	63.6–98.5%	97.3
Familial Hypercholesterolemia Pediatric Diagnostic Score (FH-PeDS)	Possible or Probable	98.4	95.5–99.7%	11.8	1.5–36.4%	92.6
	Probable	97.9	94.7–99.4%	11.8	1.5–36.4%	92.5
Inclusion criteria						91.8

V+: pathogenic variant; V−: no variant; CI: confidence intervals; MDR: mutation detection rate; HeFH: heterozygous familial hypercholesterolemia; LIPIGEN: Lipid TransPort Disorders Italian Genetic Network.

The most stringent criteria, namely those requiring higher LDL-C thresholds for a “definite” diagnosis, such as patients classified as “definite” FH according to SBR, DLCN, and FH-CAN, showed both MDR and specificity of 100%, but their sensitivity was extremely low. If molecular genetic testing had been offered only to patients fulfilling these strict definitions, only a small fraction of variant carriers would have been identified. The LIPIGEN-FH-PED score included a larger number of subjects within the “definite” category,

resulting in higher sensitivity, yet only 38.4% of variant-positive patients would have been detected.

Other criteria, such as EAS, AHA, JAS, and FH-PeDS, require lower LDL-C thresholds to classify patients into the highest probability category for HeFH diagnosis, leading to higher sensitivity but lower specificity.

The categories in which molecular testing is typically recommended include: “possible” or “definite” for SBR, “probable” or “definitive” for DLCN, “highly probable” for EAS, “HeFH diagnosis” for AHA, “probable” or “definite” for FH-CAN, “probable” or “definite” for JAS criteria and score; “probable” or “definite” for LIPIGEN-FH-PED, and “probable” for FH-PeDS, although testing should also be performed in “possible” cases for DLCN.

The MDR in these conditions was very similar to each other and to our inclusion criterion, while, using these categories as thresholds for molecular analysis would have resulted in the loss of the following proportions of variant-positive subjects: 20 (10.5%) for SBR, 107 (56.3%) for DLCN (or 26, corresponding to 13.7%, when including also “possible” cases), 12 (6.3%) for EAS, 12 (6.3%) for AHA, 14 (7.4%) for FH-CAN, 3 (1.6%) for JAS criteria and score, 12 (6.3%) for LIPIGEN-FH-PED, and 3 (1.6%) for FH-PeDS.

On the other hand, the use of criteria based on lower LDL-C thresholds would be expected to increase the number of unproductive genetic analyses; however, under the aforementioned conditions, this proportion was very similar across all criteria (range 6.8–8.1%), with the exception of the DLCN “probable” or “definite”, which did not include genetically negative subjects.

Consequently, criteria with lower cut-offs that encompass a broader population, such as JAS and FH-PeDS, resulted in minimal loss of variant-positive individuals, maintaining high MDR.f.

The inclusion of patients categorized even as “possible” FH according to DLCN, JAS score, LIPIGEN-FH-PED, and FH-PeDS increased sensitivity (reaching 100% for JAS and LIPIGEN-FH-PED, 98.4% for FH-PeDS, and 86.3% for DLCN), while reducing specificity but still preserving a high MDR.

Post hoc power analysis confirmed that the study was adequately powered to detect sensitivity values above 80% for most diagnostic criteria (power > 98% at $\alpha = 0.05$, $n = 190$), whereas the small number of V− subjects ($n = 17$) limited the statistical power to estimate specificity and for detecting moderate between-group differences in continuous variables (e.g., Cohen’s $d = 0.56$ and post hoc power = 59% for the comparison of LDL-C between V+ and V−).

4. Discussion

Several diagnostic criteria for HeFH are currently available, but no international consensus has established the superiority of any single set.

Using our inclusion criterion of LDL-C levels $\geq$ 95th percentile in both the pediatric patient (confirmed after 6 months of dietary intervention) and one parent, genetic testing for HeFH was positive in 91.8% of cases. This demonstrates a very high MDR and confirms the findings previously reported by our group in a smaller cohort [18].

Across different cohorts, approximately 5–30% of individuals with a phenotypic presentation consistent with HeFH do not carry pathogenic variants in the known genes [1]. However, Van der Graaf et al., in a study of 1430 children with LDL-C > 95th percentile and an autosomal dominant inheritance pattern of hypercholesterolemia (defined as at least one biological parent receiving treatment for hypercholesterolemia and a family history of hypercholesterolemia and CVD), reported an MDR of 95%, higher than in previous reports, likely reflecting more stringent selection criteria and more comprehensive molecular genetic analyses [27]. These findings suggest that even patients with moderately

elevated LDL-C levels, when accompanied by clear autosomal dominant transmission of hypercholesterolemia, should be evaluated for genetically confirmed HeFH.

In our study, in clinical scenarios where molecular testing would typically be performed, all evaluated diagnostic criteria showed high sensitivity and high MDR, whereas specificity remained low. Increasing specificity would require restricting molecular analysis to “definite” cases only; however, this strategy would result in unacceptably low sensitivity and a substantial number of missed variant carriers.

Regarding specific diagnostic systems, DLCN and SBR criteria are most commonly applied in adults. DLCN does not include pediatric-specific cut-offs and is therefore not directly applicable to children. In our cohort, using “probable” or “definite” HeFH according to DLCN as the threshold for molecular analysis would have resulted in missing 56.3% of variant-positive patients, making this approach unsuitable in the pediatric setting. Application of the SBR criteria led to a lower proportion of missed carriers (10.5%) compared with DLCN; nevertheless, this percentage remains clinically relevant. This limitation is related both to the minimum LDL-C cut-off of 155 mg/dL for the child and to the total cholesterol cut-off of 290 mg/dL for the parent, thresholds that may not be reached even in adults with LDL-C above the 95th percentile.

In adult populations, studies assessing the relationship between molecular findings and clinical FH diagnosis using SBR and DLCN criteria [28,29] reported high MDR and specificity only when sensitivity was low, and vice versa, reflecting the trade-off observed in our series. The main difference compared with our study is the substantially lower MDR reported in adult cohorts, likely reflecting less stringent inclusion criteria and a higher prevalence of hypercholesterolemia due to non-genetic causes, including lifestyle-related factors.

As previously shown by our group [18], the EAS criteria, and consequently the AHA criteria, which largely overlap with EAS, as well as the LIPIGEN-FH-PED criteria, result in a non-negligible proportion of missed variant carriers (6.3% across all three systems).

In the present study, we additionally evaluated FH-CAN, JAS, and the recently developed FH-PeDS criteria. FH-CAN showed a proportion of missed variant carriers of 7.4%, very similar to that observed with EAS, AHA, and LIPIGEN-FH-PED. In contrast, a key novel finding of our study is that both JAS and FH-PeDS were able to identify the vast majority of variant-positive subjects, missing only 1.6% of carriers. This improved performance appears mainly attributable to the lower LDL-C thresholds required for the child and, in part, to the lower LDL-C threshold required for the parent (180 mg/dL in JAS and any documented history of hypercholesterolemia, irrespective of precise lipid values, in FH-PeDS).

Our inclusion criteria are simpler and less time-consuming than JAS and FH-PeDS, while achieving a similarly high MDR.

Nevertheless, the key challenge remains how to optimally identify individuals with hypercholesterolemia. HeFH is markedly underrecognized worldwide [6]. Most cases are detected in adulthood through opportunistic screening, prompted either by elevated cholesterol levels or the onset of ASCVD [7], whereas diagnosis should ideally occur much earlier, given the heightened cardiovascular risk associated with cumulative exposure to elevated LDL-C levels from childhood onward. Cascade screening is widely regarded as the most practical, cost-effective, and strongly recommended strategy [1,7]. However, in routine clinical practice, parental genetic data are frequently unavailable. Selective screening based on clinical or familial risk factors (such as a family history of hypercholesterolemia or premature coronary heart disease) has also been proposed. The limitations of both cascade and selective screening include incomplete parental lipid data and negative or unreliable family histories, which are particularly common in pediatric populations. To overcome these barriers, universal pediatric lipid screening has been proposed as a

potential solution, although robust cost-effectiveness data remains limited and outcomes vary according to the LDL-C cut-offs adopted [30]. A strategy based on biochemical screening followed by molecular analysis in children with elevated LDL-C may further enhance diagnostic accuracy.

It should be noted that our cohort represents a selected population referred to a tertiary lipid center rather than a screening population; consequently, the likelihood of including children with only mildly elevated LDL-C levels is lower (in our population of 207 V+ subjects, 56 had LDL-C < 190 mg/dL, of whom 19 had LDL-C < 160 mg/dL). Studies in screening-based cohorts evaluating several of the diagnostic criteria included in our study have consistently reported lower sensitivity and MDR, but higher specificity across all evaluated criteria, as well as a substantially lower overall MDR in the population undergoing genetic testing (with significantly lower mean LDL-C levels than those observed in our study) [17,31]. This pattern has been evidenced by recent studies (in 2025) conducted in Slovenian and German populations [17,31]: the former evaluated 5-year-old children with TC $\geq$ 6.0 mmol/L ($\geq$ 232.0 mg/dL) regardless of family history, or TC $\geq$ 5.0 mmol/L ($\geq$ 193.4 mg/dL) plus a positive family history of early CVD (MDR 24.1% in the total Slovenian study population), while the latter included pediatric subjects selected solely on the basis of LDL-C $\geq$ 130 mg/dL without criteria on family history (MDR 15%).

Lowering LDL-C thresholds, as observed with these latter criteria and with our inclusion strategy, inevitably increases the risk of false positives but reduces the number of false negatives. Incorporating a specific parental criterion, as in our approach, may nonetheless limit the number of false positives. In a condition such as HeFH, in which early treatment is crucial to prevent recurrent cardiovascular events, and considering the progressively decreasing cost of genetic testing, we believe that priority should be given to maximizing the identification of affected individuals, even at the expense of a modest increase in false positives, which can subsequently be clarified through molecular analysis. Furthermore, establishing a genetic diagnosis enables cascade screening of relatives, thereby allowing treatment to be extended to additional affected family members.

In the pediatric population, LDL-C levels alone provide greater discriminatory power for FH diagnosis than in adults [32,33], owing to the reduced impact of secondary and environmental factors. Therefore, when elevated LDL-C levels are accompanied by a suggestive family history, diagnosis is generally more straightforward in childhood than in adulthood, even though clinical signs are extremely rare in pediatric patients [34]. Although family history of cardiovascular events may be informative, it is not a strongly discriminative criterion, particularly because parents are often relatively young and may already be receiving lipid-lowering therapy, with a consequent reduction in cardiovascular events.

This study has limitations that should be recognized:

1. A primary concern is that this cohort reflects a tertiary referral population rather than a general screening population. Consequently, the diagnostic performance reported here may not be directly generalizable to population-based screening settings, where LDL-C levels are typically lower and family history information is often incomplete or unavailable.
2. The inclusion criteria based on proband and parent LDL-C $\geq$ 95th percentile enriched the cohort for individuals with a high pre-test probability of FH. This likely contributed to the high MDR observed and influenced the diagnostic performance of the evaluated criteria. These aspects should be considered when interpreting the applicability of our findings to broader clinical or public health contexts. We adopted these criteria because they reflect the population typically referred to tertiary center in Italy, where universal screening has not been implemented.

3. Another limitation is the relatively small number of variant-negative individuals in our cohort. Because specificity estimates are derived from this subgroup, their precision is inherently limited and, as a consequence, comparisons of specificity across the evaluated diagnostic criteria should be interpreted with caution. Post hoc power analysis confirmed that the study was adequately powered to detect sensitivity values above 80% for most diagnostic criteria, whereas the small number of V− subjects limited statistical power both for specificity estimation and for detecting moderate between-group differences in continuous variables. Studies including larger and more heterogeneous populations are needed to provide more robust estimates of specificity.

5. Conclusions

While most established diagnostic criteria show an intrinsic trade-off between sensitivity and specificity, approaches using lower LDL-C thresholds improve the identification of variant carriers, which is critical in a condition where early treatment markedly reduces cardiovascular risk, while still maintaining a high MDR. In pediatric populations, LDL-C levels retain strong discriminatory power, and the addition of a clear parental criterion further enhances diagnostic accuracy while limiting false positives. Our simple inclusion criterion based on $\text{LDL-C} \geq 95\text{th percentile}$ in both the child and one parent yields a very high MDR, supporting its clinical utility for selecting candidates for molecular analysis. In light of the progressive reduction in genetic testing costs, and the importance of enabling cascade screening, prioritizing sensitivity to identify as many affected individuals as possible appears warranted in the pediatric setting.

Author Contributions: Conceptualization, R.B. and O.G.; methodology, R.B., C.N. and O.G.; investigation, R.B., G.M., M.F. and M.D.D.T.; data curation, R.B., C.N. and G.M.; writing—original draft preparation, R.B.; writing—review and editing, R.B., G.M., M.F., M.D.D.T., L.d.S. and O.G. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: The study was conducted according to the guidelines of the Declaration of Helsinki and approved by the Ethics Committee of Regina Margherita Children's Hospital (protocol code: 0024500, date of approval: 9 March 2016).

Informed Consent Statement: Informed consent was not requested for this retrospective study, and all data analyzed were collected as part of routine diagnosis and treatment.

Data Availability Statement: Data are available from the corresponding author upon reasonable request.

Conflicts of Interest: M.F. and M.D.D.T. are members of CEINGE-Biotecnologie Avanzate Franco Salvatore. The other authors declare no conflict of interest.

References

1. Wiegman, A.; Gidding, S.S.; Watts, G.F.; Chapman, M.J.; Ginsberg, H.N.; Cuchel, M.; Ose, L.; Averna, M.; Boileau, C.; Borén, J.; et al. European Atherosclerosis Society Consensus Panel. Familial hypercholesterolaemia in children and adolescents: Gaining decades of life by optimizing detection and treatment. *Eur. Heart J.* **2015**, *36*, 2425–2437. [\[CrossRef\]](#)
2. Akioyamen, L.E.; Genest, J.; Shan, S.D.; Reel, R.L.; Albaum, J.M.; Chu, A.; Tu, J.V. Estimating the prevalence of heterozygous familial hypercholesterolaemia: A systematic review and meta-analysis. *BMJ Open* **2017**, *7*, e016461. [\[CrossRef\]](#)
3. Beheshti, S.O.; Madsen, C.M.; Varbo, A.; Nordestgaard, B.G. Worldwide Prevalence of Familial Hypercholesterolemia: Meta-Analyses of 11 Million Subjects. *J. Am. Coll. Cardiol.* **2020**, *75*, 2553–2566. [\[CrossRef\]](#)
4. Ference, B.A.; Ginsberg, H.N.; Graham, I.; Ray, K.K.; Packard, C.J.; Bruckert, E.; Hegele, R.A.; Krauss, R.M.; Raal, F.J.; Schunkert, H.; 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*, 2459–2472. [\[CrossRef\]](#)

5. Narverud, I.; Retterstøl, K.; Iversen, P.O.; Halvorsen, B.; Ueland, T.; Ulven, S.M.; Ose, L.; Aukrust, P.; Veierød, M.B.; Holven, K.B. Markers of atherosclerotic development in children with familial hypercholesterolemia: A literature review. *Atherosclerosis* **2014**, *235*, 299–309. [CrossRef]
6. Nordestgaard, B.G.; Chapman, M.J.; Humphries, S.E.; Ginsberg, H.N.; Masana, L.; Descamps, O.S.; Wiklund, O.; Hegele, R.A.; Raal, F.J.; Defesche, J.C.; et al. European Atherosclerosis Society Consensus Panel. Familial hypercholesterolaemia is underdiagnosed and undertreated in the general population: Guidance for clinicians to prevent coronary heart disease: Consensus statement of the European Atherosclerosis Society. *Eur. Heart J.* **2013**, *34*, 3478–3490. [PubMed]
7. Tarugi, P.; Bertolini, S.; Calandra, S.; Arca, M.; Angelico, F.; Casula, M.; Cefalù, A.B.; D’Erasmus, L.; Fortunato, G.; Perrone-Filardi, P.; et al. Consensus document on diagnosis and management of familial hypercholesterolemia from the Italian Society for the Study of Atherosclerosis (SISA). *G. Ital. Dell’ Ateroscler.* **2024**, *15*, 5–32. [CrossRef] [PubMed]
8. D’Erasmus, L.; Di Costanzo, A.; Arca, M. Autosomal recessive hypercholesterolemia: Update for 2020. *Curr. Opin. Lipidol.* **2020**, *31*, 56–61. [CrossRef]
9. Scientific Steering Committee on behalf of the Simon Broome Register Group. Risk of fatal coronary heart disease in familial hypercholesterolaemia. *BMJ* **1991**, *303*, 893–896. [CrossRef]
10. World Health Organization (WHO). Familial Hypercholesterolemia (FH): Report of a Second WHO Consultation. Available online: <https://apps.who.int/iris/handle/10665/66346> (accessed on 26 February 2026).
11. Williams, R.R.; Hunt, S.C.; Schumacher, M.C.; Hegele, R.A.; Leppert, M.F.; Ludwig, E.H.; Hopkins, P.N. Diagnosing heterozygous familial hypercholesterolemia using new practical criteria validated by molecular genetics. *Am. J. Cardiol.* **1993**, *72*, 171. [CrossRef] [PubMed]
12. Gidding, S.S.; Champagne, M.A.; de Ferranti, S.D.; Defesche, J.; Ito, M.K.; Knowles, J.W.; McCrindle, B.; Raal, F.; Rader, D.; Santos, R.D.; et al. American Heart Association Atherosclerosis, Hypertension, and Obesity in Young Committee of Council on Cardiovascular Disease in Young, Council on Cardiovascular and Stroke Nursing, Council on Functional Genomics and Translational Biology, and Council on Lifestyle and Cardiometabolic Health. The Agenda for Familial Hypercholesterolemia: A Scientific Statement From the American Heart Association. *Circulation* **2015**, *132*, 2167–2192. [PubMed]
13. Ruel, I.; Brisson, D.; Aljenedil, S.; Awan, Z.; Baass, A.; Bélanger, A.; Bergeron, J.; Bewick, D.; Brophy, J.M.; Brunham, L.R.; et al. Simplified Canadian Definition for Familial Hypercholesterolemia. *Can. J. Cardiol.* **2018**, *34*, 1210–1214. [CrossRef] [PubMed]
14. Dobashi, K. Japanese Diagnostic Criteria for Pediatric Familial Hypercholesterolemia 2022. *J. Atheroscler. Thromb.* **2024**, *31*, 1026–1028. [CrossRef]
15. Watts, G.F.; Gidding, S.S.; Hegele, R.A.; Raal, F.J.; Sturm, A.C.; Jones, L.K.; Sarkies, M.N.; Al-Rasadi, K.; Blom, D.J.; Daccord, M.; et al. International Atherosclerosis Society guidance for implementing best practice in the care of familial hypercholesterolaemia. *Nat. Rev. Cardiol.* **2023**, *20*, 845–869. [CrossRef]
16. Società Italiana per lo Studio dell’ Aterosclerosi. DLCN SCORE Modificato per Pazienti Pediatrici Italiani. Available online: <http://lipigen.sisa.it/upload/LIPIGEN-FH-PED.pdf> (accessed on 26 February 2026).
17. Kafol, J.; Miranda, B.; Sikonja, R.; Sikonja, J.; Wiegman, A.; Medeiros, A.M.; Alves, A.C.; Freiburger, T.; Hutten, B.A.; Mlinaric, M.; et al. Proposal of a Familial Hypercholesterolemia Pediatric Diagnostic Score (FH-PeDS). *Eur. J. Prev. Cardiol.* **2025**, *Online ahead of print*. [CrossRef]
18. Buganza, R.; Massini, G.; Di Taranto, M.D.; Cardiero, G.; de Sanctis, L.; Guardamagna, O. Simplified Criteria for Identification of Familial Hypercholesterolemia in Children: Application in Real Life. *J. Cardiovasc. Dev. Dis.* **2024**, *11*, 123. [CrossRef]
19. Daniels, S.R.; Greer, F.R.; Committee on Nutrition. Lipid screening and cardiovascular health in childhood. *Pediatrics* **2008**, *122*, 198–208. [CrossRef]
20. Balder, J.W.; de Vries, J.K.; Nolte, I.M.; Lansberg, P.J.; Kuivenhoven, J.A.; Kamphuisen, P.W. Lipid and lipoprotein reference values from 133,450 Dutch Lifelines participants: Age- and gender-specific baseline lipid values and percentiles. *J. Clin. Lipidol.* **2017**, *11*, 1055–1064. [CrossRef]
21. World Health Organization (WHO). The WHO Child Growth Standards. Available online: <https://www.who.int/tools/child-growth-standards/standards> (accessed on 26 February 2026).
22. Rubba, P.; Gentile, M.; Marotta, G.; Iannuzzi, A.; Sodano, M.; De Simone, B.; Jossa, F.; Iannuzzo, G.; Giacobbe, C.; Di Taranto, M.D.; et al. Causative mutations and premature cardiovascular disease in patients with heterozygous familial hypercholesterolaemia. *Eur. J. Prev. Cardiol.* **2017**, *24*, 1051–1059. [CrossRef]
23. Di Taranto, M.D.; de Falco, R.; Guardamagna, O.; Massini, G.; Giacobbe, C.; Auricchio, R.; Malamisura, B.; Proto, M.; Palma, D.; Greco, L.; et al. Lipid profile and genetic status in a familial hypercholesterolemia pediatric population: Exploring the LDL/HDL ratio. *Clin. Chem. Lab. Med.* **2019**, *57*, 1102–1110. [CrossRef] [PubMed]
24. Chora, J.R.; Medeiros, A.M.; Alves, A.C.; 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*, 591–598.

25. Chora, J.R.; Iacocca, M.A.; Tichý, L.; Wand, H.; Kurtz, C.L.; Zimmermann, H.; Leon, A.; Williams, M.; Humphries, S.E.; Hooper, A.J.; et al. ClinGen Familial Hypercholesterolemia Expert Panel. The Clinical Genome Resource (ClinGen) Familial Hypercholesterolemia Variant Curation Expert Panel consensus guidelines for LDLR variant classification. *Genet. Med.* **2022**, *24*, 293–306. [\[CrossRef\]](#)
26. Buganza, R.; Nobili, C.; Massini, G.; Cardiero, G.; Di Taranto, M.D.; de Sanctis, L.; Guardamagna, O. Diagnosis of Familial Hypercholesterolemia in Children: From Clinical Features Through Gene Variants to Polygenic Score. *Genes* **2026**, *17*, 267. [\[CrossRef\]](#)
27. van der Graaf, A.; Avis, H.J.; Kusters, D.M.; Vissers, M.N.; Hutten, B.A.; Defesche, J.C.; Huijgen, R.; Fouchier, S.W.; Wijburg, F.A.; Kastelein, J.J.; et al. Molecular basis of autosomal dominant hypercholesterolemia: Assessment in a large cohort of hypercholesterolemic children. *Circulation* **2011**, *123*, 1167–1173. [\[CrossRef\]](#) [\[PubMed\]](#)
28. Damgaard, D.; Larsen, M.L.; Nissen, P.H.; Jensen, J.M.; Jensen, H.K.; Soerensen, V.R.; Jensen, L.G.; Faergeman, O. The relationship of molecular genetic to clinical diagnosis of familial hypercholesterolemia in a Danish population. *Atherosclerosis* **2005**, *180*, 155–160. [\[CrossRef\]](#)
29. Chan, D.C.; Pang, J.; Hooper, A.J.; Bell, D.A.; Bates, T.R.; Burnett, J.R.; Watts, G.F. A Comparative Analysis of Phenotypic Predictors of Mutations in Familial Hypercholesterolemia. *J. Clin. Endocrinol. Metab.* **2018**, *103*, 1704–1714. [\[CrossRef\]](#)
30. Jahn, B.; Santamaria, J.; Dieplinger, H.; Binder, C.J.; Ebenbichler, C.; Scholl-Bürgi, S.; Conrads-Frank, A.; Rochau, U.; Kühne, F.; Stojkov, I.; et al. Familial hypercholesterolemia: A systematic review of modeling studies on screening interventions. *Atherosclerosis* **2022**, *355*, 15–29. [\[CrossRef\]](#)
31. Schmieder, R.S.; Krefting, J.; Ates, S.; Schlieben, L.D.; Arens, S.; Kordonouri, O.; Sander, M.; Holdenrieder, S.; Mall, V.; Meitinger, T.; et al. Clinical scores fail to sufficiently identify children with Familial Hypercholesterolemia. *Eur. J. Prev. Cardiol.* **2025**, *Online ahead of print*.
32. Wald, D.S.; Kasturiratne, A.; Godoy, A.; Ma, L.; Bestwick, J.P.; Brewer, N.; Wald, N.J. Child-parent screening for familial hypercholesterolemia. *J. Pediatr.* **2011**, *159*, 865–867. [\[CrossRef\]](#)
33. Di Taranto, M.D.; Giacobbe, C.; Palma, D.; Iannuzzo, G.; Gentile, M.; Calcaterra, I.; Guardamagna, O.; Auricchio, R.; Di Minno, M.N.D.; Fortunato, G. Genetic spectrum of familial hypercholesterolemia and correlations with clinical expression: Implications for diagnosis improvement. *Clin. Genet.* **2021**, *100*, 529–541. [\[CrossRef\]](#) [\[PubMed\]](#)
34. Casula, M.; Gazzotti, M.; Capra, M.E.; Olmastroni, E.; Galimberti, F.; Catapano, A.L.; Pederiva, C.; LIPIGEN Group and the LIPIGEN Paediatric Group. Refinement of the diagnostic approach for the identification of children and adolescents affected by familial hypercholesterolemia: Evidence from the LIPIGEN study. *Atherosclerosis* **2023**, *385*, 117231. [\[CrossRef\]](#) [\[PubMed\]](#)

Disclaimer/Publisher’s Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.