Clinical scores fail to sufficiently identify children with Familial Hypercholesterolemia

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Abstract:

Aim: The study aimed to assess the effectiveness of three clinical diagnostic criteria (Simon Broome, MEDPED, and guideline-derived) in identifying children with familial hypercholesterolemia (FH) compared to genetic testing. The evaluation involved 1337 children with elevated LDL-C levels, focusing on the sensitivity and specificity of these clinical scores in detecting genetically confirmed FH cases.

Methods: Clinical data were gathered by a self-reporting questionnaire. Clinical FH was defined in accordance with the tested FH score. Genetically confirmed heterozygous FH (HeFH) was defined by a (likely) pathogenic variant.

Results: Of 1337 children undergoing genetic analysis, 211 showed a pathogenic FH mutation. Applying SB, MP and GL-EAS criteria resulted in 210/1337, 125/1337 and 112/835 children being categorized to have FH clinically. The sensitivity of the clinical scores ranged from 0.44-0.54 with a positive predictive value (PPV) of 0.51-0.79. The specificity was 0.91-0.97 with a negative predictive value (NPV) of 0.89-0.91. Similar results were observed for the three clinical scores regarding sensitivity, specificity, PPV and NPV in subgroup analyses defined by gender, age (<10 years vs ≥10 years), or weight (≥90th BMI-percentile vs <90th BMI-percentile).

Conclusion: Clinical FH scores offer a high degree of specificity for FH diagnosis in children, but at the expense of low sensitivity. Specifically, half of the mutation-positive children in this study would have been missed for early diagnosis and preventive treatment. Given the widespread availability of affordable genetic testing such analysis should be performed at a lower threshold than that indicated by these clinical scores.

Lay summary:

Commonly used clinical scoring systems (Simon Broome, MEDPED, GL-EAS) for the diagnosis of familial hypercholesterolemia (FH) were evaluated in the pediatric VRONI cohort using genetically confirmed heterozygous FH cases as reference. Clinical scores alone were found to be insufficient, strongly supporting routine use of genetic testing.

- All clinical FH scores tested showed high specificity (up to 97%) but low sensitivity (44-54%), missing approximately half of children with genetically confirmed FH.
- Genetic analysis in case of elevated LDL cholesterol can significantly improve the detection rate of FH and enables early preventive treatment as well as detection of other affected family members through cascade screening.

Key words: Familial Hypercholesterolemia, screening, genetic testing, clinical scores, prevention

Introduction

Familial hypercholesterolemia (FH) is a genetic condition characterized from birth by abnormally high serum levels of low-density lipoprotein cholesterol (LDL-C). Since identification of the low-density lipoprotein receptor gene (*LDLR*), thousands of mutations have been implicated as being causal. Most pathogenic variants for FH are inherited autosomal-dominantly and found in *LDLR* (>80%), followed by variants in the *APOB* and *PCSK9* genes. Heterozygous FH is listed as the most frequent monogenic disorder with a presumed prevalence of around 0.2 - 1.0%.[1-3] FH patients carry a high cardiovascular disease (CVD) risk. Fifty percent of affected men experience a cardiovascular event by the age of 50 years, and 30% of women do so by the age of 60 years.[4] Homozygous FH is uncommon (1: 500.000) but CVD advances even more rapidly and with greater severity.[1-3]

The LDL-C burden and the correlated lifetime risk of death from CVD can be reduced by 75% through early detection and treatment.[5] However, systematic screening strategies are inconsistently implemented leading to FH being massively underdiagnosed and undertreated.[5-8] Importantly, lipid-lowering therapy in children was evaluated only in those with a genetically confirmed diagnosis.[5,9] Therefore, a global challenge is to identify children with FH and to ensure the diagnosis by a genetic test prior to initiation of pharmacotherapy.

In adults, FH can be diagnosed clinically based on laboratory parameters, family history and physical characteristics. In childhood the diagnosis represents a complex task because of the lack of symptoms or clinical signs, although it is the optimal period for discrimination between FH and non-FH using LDL-C measurements.[10] Several FH scoring systems based on clinical criteria are currently available. The “Make Early Diagnosis to Prevent Early Deaths” (U.S. MEDPED) Score [11], the Simon Broome criteria [12] or the guideline-derived (GL-EAS) [13] criteria are based on the LDL-C value and the family's medical history. The Dutch Lipid Clinic Network (DLCN) score also

incorporates physical characteristics such as tendon xanthomas.[14] It is worth noting that the DLCN criteria are not validated in children [15], whereas other criteria such as Simon Broome scoring might be more appropriate as they contain specific cut-off levels for LDL-C in this specific age group.

However, the use of diagnostic scores in children is poorly implemented, missing an opportunity for early prevention of ASCVD. In addition, it is not clear whether the performance of the proposed clinical diagnostic scores can efficiently detect children with FH. False-negative clinical score test results not only miss the opportunity for early preventive treatment of the screened child, but also the opportunity to identify the affected parent through reverse cascade screening. In addition, a negative clinical score result in childhood may delay or even prevent a correct diagnosis of FH in adulthood. It is therefore important to understand the quality and limitations of commonly used clinical FH scores.

The present study evaluated the performance of clinical diagnostic criteria in children with a genetic diagnosis of FH enrolled in the Vroni Study and addressed the following question: How many children with genetically confirmed FH are missed by using only clinical criteria alone? To our knowledge, this is the first study to compare clinical FH scores with dedicated gene sequencing in children with high LDL-C levels.

METHODS

We evaluated the performance of established FH scoring systems within the Vroni Study cohort, based on clinical characteristics and genotype data. We applied clinical criteria of the Simon Broome Register Criteria (SB), the Make Early Diagnosis to Prevent Early Death Criteria (MEDPED), and the guideline-derived (GL-EAS) criteria separately, thus comparing the clinical diagnosis of FH with genetically confirmed (heterozygous) FH (HeFH). Additionally, different LDL-C cut-off values were also analyzed in terms of test performance regarding HeFH detection.

Patient Cohort

The design and methods of the Vroni Study have been previously described in detail [16]. Vroni is a FH screening program for children aged 5 to 14 in Bavaria, conducted during regular pediatric visits. The process involves central laboratory measurements of EDTA blood samples (200 µl capillary or 1.2 ml venously) and, in case of LDL-C ≥130 mg/dl (3.37 mmol/l), also genetic analysis via a dedicated FH panel. Clinical data is gathered via questionnaires, including self-reporting on family history (defined as atherosclerotic cardiovascular disease (ASCVD) events occurring before the age of ≤55 years for men and ≤60 years for women) and physical examination data. Cases with missing genetic data or clinical data for calculation of the scores (details see below) were excluded from the study cohort.

Laboratory and Genetic Analyses

Laboratory tests were carried out at the Institute for Laboratory Medicine at the German Heart Centre in Munich, TUM University Hospital. The cholesterol measurements were performed in K3 EDTA plasma (after centrifugation of EDTA blood samples) on a Roche cobas c 501 instrument, utilizing icterus, hemolysis, and lipemia indices for quality control. Residual blood clots were resuspended in 100 µl 1x phosphate-buffered saline and stored at -80°C in individual 300 µl 2D code FluidX Cryo Tubes.

Genetic tests were carried out at the Institute of Neurogenomics (ING) at the Helmholtz Zentrum in Munich. After DNA extraction from the resuspended blood clots, sequencing was performed on a Illumina NovaSeq 6000 utilizing a dedicated Next-Generation-Sequencing (NGS) panel by TWIST Bioscience. The customized FH-panel encompasses exonic regions of 23 genes associated with lipid metabolism (**Supplemental Table 1**), particularly the entire genomic region of the *LDLR*, *APOB* and *PCSK9* and Panel sequencing provides at least a 1000-fold coverage of the target region. For analyses reads are mapped to the human genome build GRCh37/hg19 and for interpretation ClinVar, GnomAD and the TUM Exome Variant Annotation Database are utilized. Genetic variants with an allele frequency of less than 0.1% were classified as 'pathogenic' if listed in ClinVar as 'likely_pathogenic' or 'pathogenic', or if assessed as 'likely_pathogenic' or 'pathogenic' according to the published American College of Medical Genetics criteria, or if predicted as a loss-of-function variant (stop, frameshift, canonical splice-site variants or larger deletions). Carriers with these variants were defined as monogenic FH and all other cases defined as genetically negative for the purposes of this analysis.

Clinical Scores

Table 1 provides a detailed overview of the criteria used to define clinical FH based on the Simon Broome Register Criteria (SB), the MEDPED Criteria (MP) and the Guideline-derived criteria (GL-EAS).

Simon Broome Criteria Score (SB) was positive for (probable) FH if (a) LDL-C was >155 mg/dl or total Cholesterol (TC) was >260 mg/dl and (b) the family history was either positive for premature coronary heart disease (CHD) in parents/grandparents or for hypercholesterolemia (LDL-C >160 mg/dl) in siblings or parents. For those aged 16 and over, the corresponding cut-off values were 190 mg/dl for LDL-C and 290 mg/dl for TC.

MEDPED Criteria Score (MP) was positive for FH if (a) TC was >270 mg/dl or LDL-C >200 mg/dl or if (b) TC >220 mg/dl plus one parent with FH or LDL-C >155 mg/dl plus one parent with FH.

Guideline-derived Score (GL-EAS) was positive for FH in case of: (a) LDL-C >190 mg/dl on both the first and second blood samples, (b) LDL-C >160 mg/dl on both samples and parental history of either premature coronary heart disease or hypercholesterolemia (LDL-C >160 mg/dl), or (c) LDL-C >135 mg/dl on both samples and one parent diagnosed with monogenic FH.

Statistical Analyses:

To test the performance of the three scoring systems (SB, MP and GL-EAS) analysis was carried out in all genetically tested patients. Continuous variables are presented as mean ± SD, whereas categorical variables are presented as cases (n) and percentage rate (%).

For each tool, we calculated the key performance metrics: sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and also Cohen's Kappa. These metrics were derived from basic diagnostic test results: true positives (TP), false positives (FP), true negatives (TN), and false negatives (FN). To assess differences in baselines between different groups, two statistical tests were conducted. The Independent Samples t-Test for normal distribution and equal variances or the Mann-Whitney U Test serves as a non-parametric alternative for non-normal distribution. All statistical analyses and visualizations were conducted using Python version 3.9.16.

RESULTS

Baseline characteristics

As of March 2024, a total of 17,196 cases have been screened in the Vroni Study and children with LDL-Cholesterol (LDL-C) ≥130 mg/dl were scheduled for genetic analysis according to the study protocol. For this study only children (i.e. <18 years) with genetic data were included and thus 15,859 cases excluded. Of the 1,337 (7.8%) cases with genetic analysis, all had sufficient clinical data to calculate at least one of the clinical scores and were therefore included in this study. For the calculation of the GL-EAS, only the subgroup of 835 children with data on a second LDL-C measurement were analyzed. Overall, mean age was 11.3 ± 3.4 years (mean ± SD),mean LDL-C level was 146.5 ± 28.8 mg/dl and 53.9% were female.

Genetic data showed a pathogenic FH variant in 211 cases (15.8%). All children were heterozygous for the FH variant (HeFH), i.e. we observed no case of homozygous or compound heterozygous FH. Children with HeFH had higher LDL-C (177.3 ± 43.2 mg/dl vs. 140.7 ± 20.7 mg/dl, p<0.001) and TC (254.2 ± 46.2 mg/dl vs. 222.1 ± 28.5 mg/dl, p<0.001) levels, as well as a lower weight z-score, BMI z-score and a lower proportion of overweight children (BMI >90th percentile) compared with cases without pathogenic mutations. In terms of age, sex and height z-score no significant difference was observed between children with and without pathogenic FH variants (**Table 2**). Within the HeFH group 83.4% (176/211) of pathogenic variants related to the *LDLR* gene and 16.1% (34/211) to the *ApoB* gene. Individuals with a *LDLR* variant had significantly higher TC (257.0 ± 48.5 vs. 236.7 ± 34.5 mg/dl; p = 0.033) and numerically higher LDL-C (178.7 ± 44.0 vs. 165.1 ± 37.8 mg/dl, p = 0.077) levels compared to carriers of ApoB mutations.

Performance of clinical scores

Clinical FH according to the Simon Broome (SB) probable FH criteria (SB_FH) was present in 210 (15.7%) of the 1337 children, giving a sensitivity of 0.51, a specificity of 0.91, a positive predictive value (PPV) of 0.51 and a negative predictive value (NPV) of 0.91 **(Figure 1 and 2)**. Using the

MEDPED (MP) criteria 125 (9.3%) of 1337 children had clinical FH (MP_FH) and the test parameters were 0.44 and 0.97 for sensitivity and specificity, 0.74 for PPV and 0.90 for NPV **(Figure 1 and 2)**.

Because the Guideline-derived (GL-EAS) FH criteria require two LDL measurements at different time points, only a subset of our cohort (835/1337) provided sufficient clinical data (i.e. two LDL data points). The subgroup had slightly higher TC and LCL-C levels compared to the overall cohort and 112 (13.4%) fulfilled all criteria for clinical FH (GL-EAS _FH), giving a sensitivity of 0.54, a specificity of 0.97, a PPV of 0.79 and a NPV of 0.89 **(Figure 1 and 2)**. The baseline characteristics of this study cohort and the various subgroups are shown in **Table 2**. Focusing on children who were positive for both, i.e. genetic analysis and one of the clinical scores, the mutation site for true positives of SB was the *LDLR* gene in 83.2% (89/107) and the *ApoB* gene in 15.9% (17/107). Correspondingly, in MP it was 85.9% (79/92) *LDLR* and 13.0% (12/92) *ApoB* gene, and in GL-EAS 85.4% (76/89) and 14.6% (13/89), respectively. Overall this is similar to the ratio of the whole HeFH group.

Subgroup analyses

For further comparison, we also analyzed a LDL threshold of 170 mg/dl as a single criterion, i.e. without further clinical data. This LDL-C threshold 170 mg/dl (LDL-C >170 mg/dl) performed similar to the three clinical scoring systems (**Figure 1 and 2**) with a sensitivity of 0.52, a specificity of 0.93, a PPV of 0.59 and a NPV of 0.91. Regarding overlap of true positives **Figure 3** visualizes the cross-section of LDL-C >170 mg/dl, SB and MP (GL-EAS excluded due to reduced cohort).

For sex, sensitivity was slightly higher (0.46-0.58 vs. 0.41-0.51) and PPV lower (0.48-0.77 vs. 0.54-0.82) in the female subgroup, with no differences in specificity or NPV.

For age (<10 vs. ≥10 years), the results showed more variation, with MP and GL-EAS scores showing a higher detection rate in the age group <10 years with sensitivities of 0.49 and 0.58 vs.

0.41 and 0.52, while SB was nearly identical (0.49 vs 0.51), respectively. All clinical scoring systems performed similarly between age groups in terms of specificity, PPV and NPV.

Among children with elevated LDL-C levels, 16.5% (220/1337) were overweight, defined as BMI ≥90th percentile. Within this small subgroup, the sensitivity of the scoring systems showed heterogenous results (range 0.26-0.53), with no major differences in specificity, PPV or NPV. The subgroup <90th BMI percentile had the same levels of test quality criteria as the overall cohort.

In addition, Cohen's kappa was calculated for the clinical scores in the primary sample and in each of the subgroups (see Supplementary Table 2). No substantial differences were observed between the three clinical scores or between the different subgroups. Cohen's kappa ranged from 0.47 to 0.57 in the study cohort, indicating moderate agreement between the clinical scores evaluated and the genetic results. For the subgroups, the range was wider, from 0.42 to 0.60 (excluding the small subgroup with BMI ≥90th percentile), but also indicative of moderate agreement. The overall results suggest that the scores are broadly consistent and that there is no significant increase in sensitivity by calculating multiple scores for the same individuals, particularly in relation to increased workload. The plots and heat maps of the subgroup analyses can be found in the **Supplemental Material**.

DISCUSSION

Analyzing children beyond the 92nd percentile of the LDL-C distribution we demonstrate a critical gap between clinically and genetically diagnosed FH cases. Specifically, the performance of the Simon Broome Register Criteria (SB), probably the most widely used pediatric FH score, the MEDPED Criteria (MP) and Guideline-derived Criteria (GL-EAS) reached good specificity (0.91-0.97) and NPV (0.89-0.91). However, the overall sensitivity ranged from 0.44 to 0.54, with a maximum sensitivity of 0.58 for the GL-EAS in the subgroup of children <10 years and a minimum sensitivity of 0.41 for the MP in the subgroup of children ≥10 years. Thus, clinical scores miss about half of cases carrying a FH mutation in our German pediatric cohort, consistent with previously published data in children as well as in adults.

For example, the recently published cross-sectional study on FH by the European Atherosclerosis Society Familial Hypercholesterolaemia Studies Collaboration showed that half of the monogenic FH cases would be misdiagnosed as negative according to clinical scores. In adults the performance of the commonly used DLCN (Dutch Lipid Clinic Network) criteria were examined in the British Columbia FH Registry (n=626), showing that the proportion of monogenic FH was 37% in the 'probable FH' group and 74% in the 'definite FH' group.[17] In terms of sensitivity, the DLCN score classified 28.5% of 1377 adults with monogenic FH from the Italian LIPIGEN study as "probable FH" and 37.9% as "definite FH".[18] Another study from the USA retrospectively applied the DLCN and MEDPED criteria to adults with monogenic FH (n=229) and found that only 23.7% and 24.7%, respectively, were correctly identified as FH cases via clinical scores.[19] Other published data corroborate these results and show a clear limitation of the clinical FH scores, in terms of specificity (range ca. 30-80%, depending on the scientific group and clinical score) but more so in terms of sensitivity (range ca. 25-66%).[20-23]

The pitfall of using clinical FH scores as a screening tool is the low sensitivity as they incorrectly classify FH cases with a less pronounced phenotype as ‘healthy’, who are nevertheless at increased cardiovascular risk. Bellows et al. published data, that only 25% of genetically identified

FH cases would also meet clinical DLCN criteria alone.[24] The diagnostic yield in young adults (aged 20-39 years) was 3.5 FH cases per 1000 adults for genetics alone and 1.3 FH cases with the DLCN criteria alone. Thus, in the most relevant adult subgroup for cardiovascular primary prevention, the DLCN score was estimated to identify only one in four monogenic FH cases and to miss the other three.[25] Our data support the conclusion that clinical scoring systems also have a low sensitivity in children and perform almost identically to a simple LDL-C cut-off value at 170 mg/dl. The latter may partly be attributable to a less pronounced phenotype during the early stages of FH. Recent research also supports the notion that a positive family history has limited predictive value in identifying FH, as known genetic factors explained only 22% of the likelihood of a positive family history of premature CHD.[26]

In addition to the variable expression of the phenotype (inter-individually and age-dependent), clinical FH scoring systems are also limited by their reliance on subjective information, such as personal and family cardiovascular history, which may be incomplete or biased. Missing or incorrect data may lead to underdiagnosis of FH when relying on clinical scores. Overall, less than half of the children in Vroni Study meet the clinical criteria for probable or definite FH, which is consistent with the previously published data. Taking these results together with the reduced cost and wide availability of NGS, there is a strong case for routine genetic testing in children with high LDL-C levels. Only in cases where genetic testing is not available or is actively refused by families (e.g. due to fear of insurance consequences of a positive test) should clinical scores be used for diagnosis. However, even then it is important to be aware of the limitations of currently used scoring systems. An example of a successfully established universal FH screening program is Slovenia. [27] Genetic analysis is always performed from a total cholesterol level of 232 mg/dl [6 mmol/l], which is approximately the 99th percentile in 5-year-olds and explains the high reported diagnostic yield. Applied to the Vroni study, this would correspond to an LDL threshold of 170 mg/dl, and our data show a sensitivity of 52% for the LDL-170 score. Therefore, it can be assumed

that by lowering the cut-off, up to twice as many index cases could be identified in Slovenia at the expense of a lower yield.

Overall, we strongly advocate that genetic testing should be indicated at a low threshold (e.g. LDL-C above the 95th percentile), which is well below that used in the currently established clinical scores. This is based on our own and published data showing that clinical scores often miss FH, particularly in children and young adults, and thus miss the opportunity for timely preventive measures. To improve test specificity, genetic analysis can be added in patients with 'clinical FH' to detect underlying mutations and verify the FH diagnosis.

For affected individuals, genetic testing can also provide an understandable explanation for possible premature cardiovascular events in the family. Moreover, the genetic diagnosis provides a clear rationale for starting a lipid-lowering therapy (LLT) even in children after the age of six.[13] The clear and reliable diagnosis and recommendation derived from genetic testing not only motivates affected children and families to make lifestyle changes and start lipid-lowering therapy at the outset, but is also crucial for long-term motivation.[27,28]

In addition, genetic testing facilitates familial cascade screening, allowing the (early) identification of other first-degree relatives in a reliable manner, even in the case of less pronounced phenotypes and has been shown to improve cascade screening in many countries.[29,30] Implementing a universal screening program in children to identify index cases, combined with reverse cascade screening of family members, can result in the diagnosis of half of all FH cases within 19 years.[31]

In terms of cost-effectiveness, there are data for both universal FH screening and cascade screening that demonstrate cost-effectiveness from a health economic perspective.[8,32-34] Moreover, combination with a reverse cascade screening offers affected family members the benefits of early intervention, reducing the risk of cardiovascular events (e.g. myocardial infarction and/or cardiac death) and helping children to grow up in a healthy family environment. The introduction of routine genetic testing must be undertaken with particular care and attention to

national ethical and legal implications, as well as the need for genetic counselling. In addition, the logistical challenges, not only for primary testing but also for long-term follow-up of positive children, need to be considered. As health care systems and established medical infrastructure vary widely from country to country, the implementation of FH screening is best discussed within national health committees and professional associations of pediatricians, cardiologists, and pediatric cardiologists, and adapted nationally to cultural and ethical backgrounds. These considerations are essential for successful implementation, as well as for managing expectations and ensuring informed decision making. Slovenia has already implemented a universal screening program, and in Germany the Vroni study may serve as a blueprint for universal FH screening.

Finally, knowledge of the specific genetic mutation may help to tailor the LLT in terms of optimizing treatment efficacy through choice of drug class and in terms of treatment goal by predicting disease severity.[35] For example, nonsense mutations in the LDLR gene are often associated with higher LDL-C levels than missense mutations,[28,36] and the gene involved may also predict disease severity; APOB and PCSK9-related FH phenotypes are generally less severe than LDLR phenotypes and, in general, monogenic FH is linked to a more severe form of preclinical atherosclerosis in the carotid and coronary arteries compared with cases of hypercholesterolemia with a polygenic origin.[17,37,38] In terms of LLT, ezetimibe is significantly more effective in cases of gain of function variants in the Niemann-Pick C1-like 1 (NPC1L1) protein.[39]

LIMITATIONS AND STRENGTHS

Our analyses have several limitations: (a) the study's reliance on standardized referral forms introduces potential biases in data accuracy and completeness, particularly regarding family history and clinical symptoms and (b) a proportion of cases were excluded due to missing data for the calculation of the clinical scores. However, these limitations may also reflect the “real world setting” in pediatric outpatient clinics. (c) Enrollment is performed by local pediatricians and thus

a preselection bias cannot be rolled out. (d) Clinical scores could not be exactly replicated, as e.g. family history for hypercholesterolemia was only yes/no for above 160 mg/dl, thus performance, especially sensitivity, may be overestimated. (e) Cases with genetic variants classified as VUS were treated as 'genetically negative', which may have misclassified some cases. (f) We have no follow-up data to demonstrate a higher risk in children with a genetic versus with clinical diagnosis of FH. (g) The clinical scores analyzed were designed for use in children, but to our knowledge no large validation studies have been published in children. However, these scores are universally used and accepted as a clinical tool.

The strength of our study is based on population-wide screening of a large number of children under standardized conditions. All children with LDL-C above the 92nd percentile were considered. Moreover, we used a dedicated FH panel on a NGS platform that comprehensively covers a wide range of genes associated with dyslipidemia and thus provides solid data quality for the identification of HeFH.

CONCLUSION

Although clinical FH scores are easy to perform, the present study demonstrates a major limitation of these scores, i.e. a low sensitivity in children. With clinical scores alone, half of the monogenic FH cases in a large population-based pediatric cohort would have been missed. Considering the wide availability and greatly reduced cost of next-generation sequencing, we propose that genetic testing should be implemented as an integral part of routine diagnostics to significantly improve the identification of FH cases, allowing for precise early preventive intervention to combat he risk of premature ASCVD.

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Authors contribution

HS, VS and RSS set the framework and conception of the study. RSS and VS drafted the manuscript. WK and HS provided intellectual input and critically reviewed the manuscript. Statistical analysis was performed by JK. All authors contributed to the acquisition, analysis, and interpretation of the underlying VRONI data. All authors gave final approval and agreed to be accountable for all aspects of the work, ensuring its integrity and accuracy.

Data availability

Individual level data are not publicly available due to its sensitive nature. To gain access to pseudonymized data in accordance with the consent of the study, data requestors will need to contact the VRONI main office in Munich as well as sign a data access and use agreement. All bioinformatics applications can be requested at the VRONI main office in Munich from the corresponding authors

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Tables

Table 1	Simon Broome (SB)	MEDPED	GL-EAS
FH CRITERIA	(A) LDL-C >155 mg/dl OR TC >260 mg/dl * AND Family history positive for premature CHD in parents/grandparents OR hypercholesterolemia (LDL-C >160 mg/dl) in siblings/parents * for age >16 years LDL-C >190mg/d OR >TC >290mg/dl	(A) TC >270 mg/dl OR LDL-C >200 mg/dl (B) TC >220 mg/dl OR LDL-C >155 mg/dl AND one parent with FH	(A) LDL-C >190 mg/dl in 1st and 2nd sample (B) LDL-C >160 mg/dl in 1st and 2nd sample AND one parent with premature CHD OR with hypercholesterolemia (C) LDL-C >135 mg/dl in 1st and 2nd sample AND one parent with FH

Table 1: Listing the clincal criteria used to determine Clinical FH for each of the tested Scores: Simone Broome (SB), MEDPED (MP), Guideline Criteria (GL-EAS).

Table 2	HeFH (n=211)	No FH (n=1126)	p-value
Age (years)	11.3 (± 3.5)	11.3 (± 3.4)	0.759
Sex (male)	103 (48.9%)	513 (45.6%)	0.426
Height (cm)	139.6 (± 20.9)	139.3 (± 19.4)	0.649
Height z-score	-0.14 (± 0.93)	-0.14 (± 1.01)	0.923
Weight (kg)	37.6 (± 16.8)	39.7 (± 18.5)	0.219
Weight z-score	-0.12 (± 0.86)	0.07 (± 1.00)	0.019
BMI (kg/m²)	18.3 (± 3.9)	19.3 (± 5.0)	0.033
BMI z-score	-0.07 (± 0.72)	0.14 (± 0.88)	<0.001

Overweight BMI ≥90th percentile	19 (9.0%)	201 (17.9%)	0.002
Total Cholesterol (mg/dl)	254.2 (± 46.2)	222.1 (± 28.5)	<0.001
LDL-C (mg/dl)	177.3 (± 43.2)	140.7 (± 20.7)	<0.001
HDL-C (mg/dl)	53.4 (± 9.9)	59.0 (± 14.1)	<0.001
Gene location of FH mutation	LDLR: 176 (83.4%) APOB: 34 (16.1%) LPL: 1 (0.5%)	n. a.	

Table 2: Baseline Criteria of this study cohort split into Heterozygous FH group (HeFH) and those without a (likely) pathogenic mutation (No FH). Variables are either mean (± SD) or absolute case-numbers (percentage).

Figures

Figure 1: Visuel representation of Sensitivity on the left, followed by Specificity, positive predictive value (PPV) and negative predictive value (NPV) for each clinical Score tested. SB is represented in blue, MP in orange, GL-EAS in green and LDL-170 score in red.

Figure 2 A-D: Heatmaps of (A) Simon Broom Criteria Score (SB), (B) MEDPED Criteria Score (MP), (C) Guideline-derived Score (MP) and (D) LDL-170 Score. All heatmaps compare HeFH vs the respective clinical scoring system with Clinical FH in the upper row and cases not meeting clinical Criteria in the lower row, as well as heterozygous FH in the left column and cases without (likely) pathogenic FH mutation in the right column.

Figure 3: Venn-diagramm on the Overlap of HeFH cases (i.e. true positives) identified by SB (upper left), MP (upper right) and simple LDL-cut-off at 170 mg/dl (lower middle).

Supplemental Material:

Supp. Table 1: List of the 23 genes covered by the NGS targeted FH panel

Supp. Table 2: Cohen's Kappa values for all clinical scores in main cohort and all subgroups

Supp. Figure 1: Bar graph of the test quality criteria in the subgroups male (left) vs. female (right). The graph shows the Sensitivity, Specificity, PPV and NPV for SB in blue, MP in orange and GL-EAS in green.

Supp. Figure 2: Heatmaps of SB, MP and GL-EAS (left to right) in the subgroups male (left) vs. female (right). Heatmaps compare HeFH vs Clinical FH according to the scoring system. HeFH cases are in the left column and cases without FH mutation in the right column. Clinical FH cases are in the upper row and cases not meeting clinical Criteria in the lower row.

Supp. Figure 3: Bar graph of the test quality criteria in the subgroups <10 years (left) vs. ≥10 years (right). The graph shows the Sensitivity, Specificity, PPV and NPV for SB in blue, MP in orange and GL-EAS in green.

Supp. Figure 4: Heatmaps of SB, MP and GL-EAS (left to right) in the subgroups <10 years (left) vs. ≥10 years (right). Heatmaps compare HeFH vs Clinical FH according to the scoring system. HeFH cases are in the left column and cases without FH mutation in the right column. Clinical FH cases are in the upper row and cases not meeting clinical Criteria in the lower row.

Supp. Figure 5: Bar graph of the test quality criteria in the subgroups <90th BMI-percentile (left) vs. ≥90th BMI-percentile (right). The graph shows the Sensitivity, Specificity, PPV and NPV for SB in blue, MP in orange and GL-EAS in green.

Supp. Figure 6: Heatmaps of SB, MP and GL-EAS (left to right) in the subgroups <90th BMI-percentile (left) vs. ≥90th BMI-percentile (right). Heatmaps compare HeFH vs Clinical FH according to the scoring system. HeFH cases are in the left column and cases without FH mutation in the right column. Clinical FH cases are in the upper row and cases not meeting clinical Criteria in the lower row.

Graphical abstract: The graphical abstract depicts the findings of this study, which was conducted on a subset of 1,337 cases that underwent genetic analysis out of 17,196 children who were screened for familial hypercholesterolemia (FH) in the VRONI Study. Genetic testing identified 211 cases of familial hypercholesterolemia (FH). In comparison, established clinical scores (Simon Broome, MEDPED, and Guideline score) demonstrated a high specificity of 94%. However, at the cost of a low sensitivity of approximately 50% for in identifying monogenic FH in children.

Figure 1

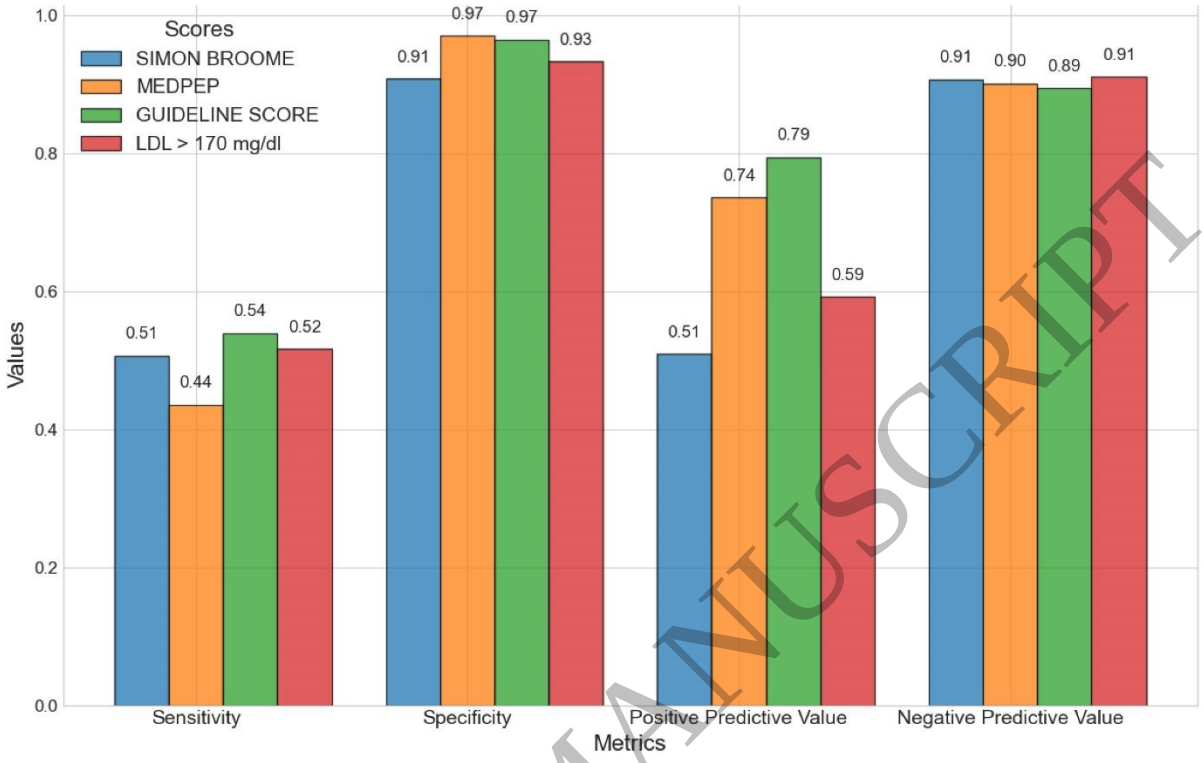

Figure 1
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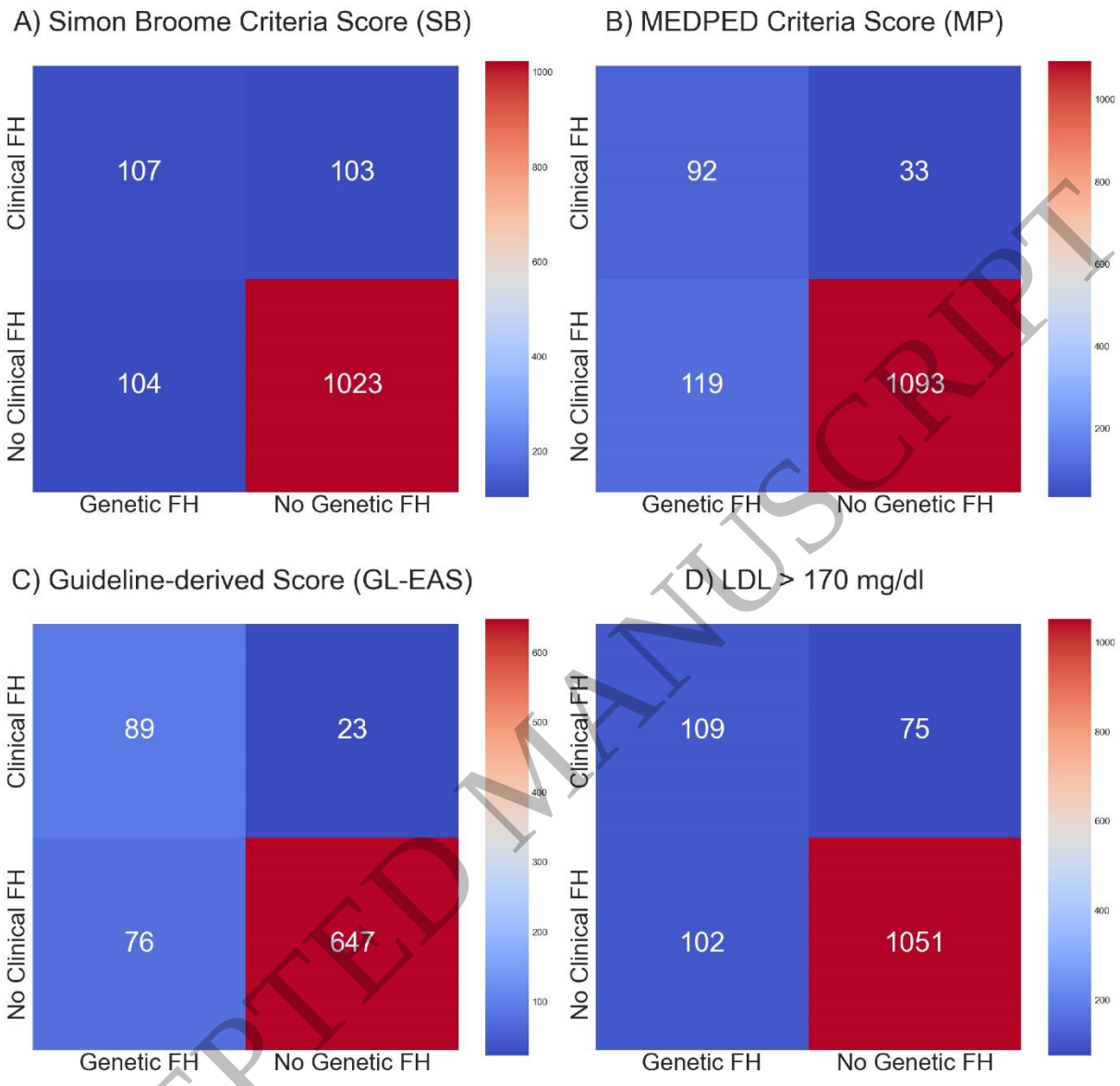

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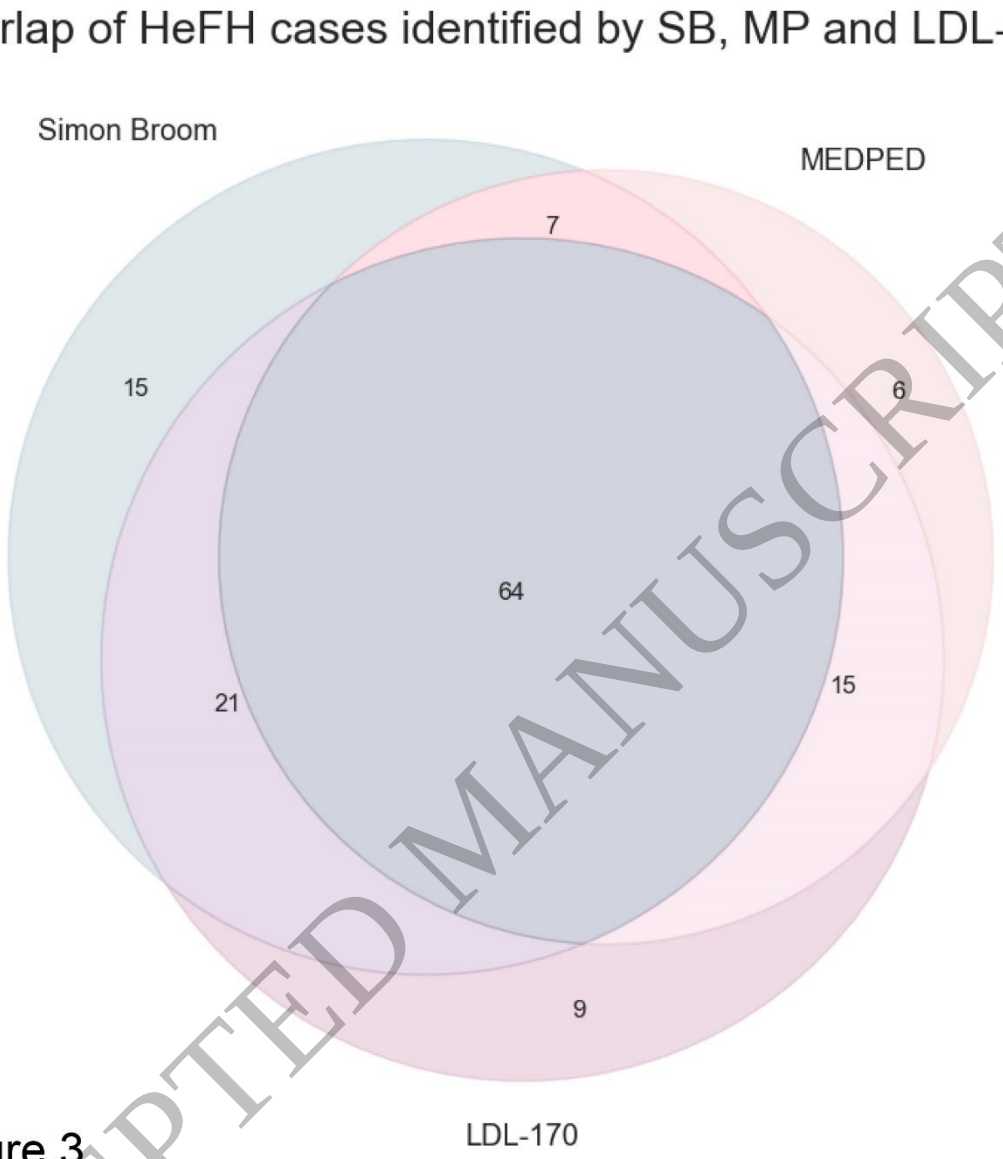

Figure 3

Figure 3
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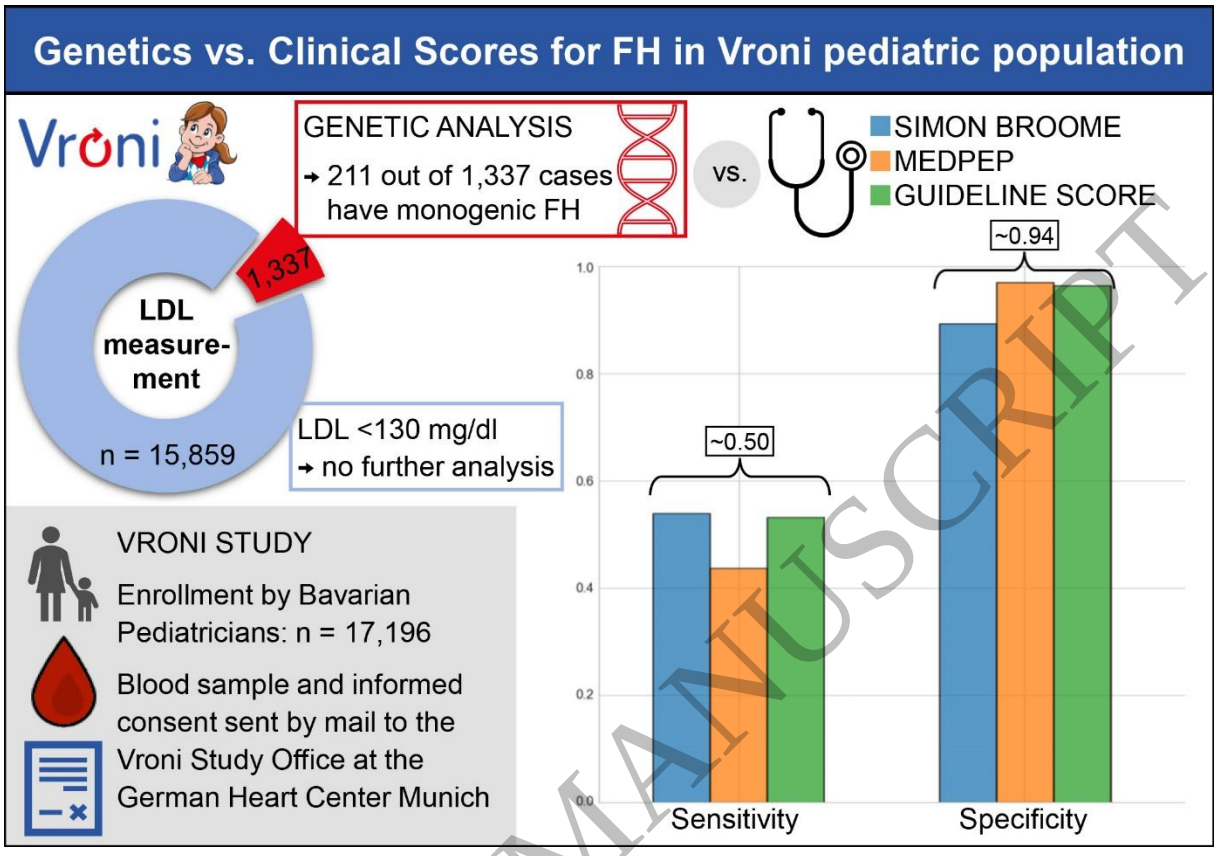

Graphical Abstract