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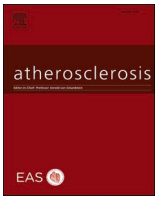

Enhanced identification of familial hypercholesterolemia using central laboratory algorithms☆

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ABSTRACT

Background and aims: Familial hypercholesterolemia (FH) is a highly prevalent genetic disorder resulting in markedly elevated LDL cholesterol levels and premature coronary artery disease. FH underdiagnosis and undertreatment require novel detection methods. This study evaluated the effectiveness of using an LDL cholesterol cut-off ≥99.5th percentile (sex- and age-adjusted) to identify clinical and genetic FH, and investigated underutilization of genetic testing and undertreatment in FH patients.

Methods: Individuals with at least one prior LDL cholesterol level ≥99.5th percentile were selected from a laboratory database containing lipid profiles of 590,067 individuals. The study comprised three phases: biochemical validation of hypercholesterolemia, clinical identification of FH, and genetic determination of FH.

Results: Of 5614 selected subjects, 2088 underwent lipid profile reassessment, of whom 1103 completed the questionnaire (mean age 64.2 ± 12.7 years, 48% male). In these 1103 subjects, mean LDL cholesterol was 4.0 ± 1.4 mmol/l and 722 (65%) received lipid-lowering therapy. FH clinical diagnostic criteria were met by 282 (26%) individuals, of whom 85% had not received guideline-recommended genetic testing and 97% failed to attain LDL cholesterol targets. Of 459 individuals consenting to genetic validation, 13% carried an FH-causing variant, which increased to 19% in clinically diagnosed FH patients.

Conclusions: The identification of a substantial number of previously undiagnosed and un(der)treated clinical and genetic FH patients within a central laboratory database highlights the feasibility and clinical potential of this targeted screening strategy; both in identifying new FH patients and in improving treatment in this high-risk population.

1. Introduction

Familial hypercholesterolemia (FH) is the most prevalent autosomal genetic disorder in lipoprotein metabolism, in which affected individuals are exposed to increased levels of low-density lipoprotein (LDL) cholesterol from birth onwards, leading to the accelerated onset of atherosclerotic cardiovascular disease (ASCVD) [1]. FH is estimated to affect 1 in 300 individuals, with an estimated global prevalence of 30 million subjects [2]. The high prevalence and the 2- to 26-fold increase in premature ASCVD risk make FH a significant threat to both individual and public health [3].

Early identification of FH followed by potent lowering of LDL cholesterol is fundamental to effectively reduce the lifetime cumulative cholesterol burden and associated ASCVD risk [4–6]. Regrettably, current global estimates indicate that over 90% of FH patients remain undiagnosed, leading to significant undertreatment of this population,

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emphasizing the need for more effective FH screening and detection methods [6–9]. Various strategies to improve the diagnostic rates of clinical and genetic FH have been proposed, including opportunistic, selective, systematic and universal screening [9–11]; although the most (cost-)effective approach to identify new FH patients remains to be determined. Opportunistic screening of extensive databases, such as population biobanks and large cohorts of blood donors, has been reported [12,13], but the yield of these strategies was limited [9]. Employing a more targeted approach, such as selecting patients from a centralized laboratory database based on sex- and age-adjusted LDL cholesterol levels, holds the promise to increase the yield of identifying FH patients.

The present study aimed to assess the effectiveness of employing an LDL cholesterol cut-off exceeding or equal to the 99.5th percentile for sex and age in identifying undiagnosed clinical and/or genetic FH cases in a central laboratory dataset encompassing lipid profiles of 600,000 subjects in the Netherlands. In parallel, this study evaluated the extent of underutilization of genetic testing as well as undertreatment in clinically and/or genetically diagnosed FH patients.

2. Patients and methods

2.1. Study design and participants

In this cross-sectional study, participants were selected from a laboratory database located within the Atalmedial Medical Diagnostic Centers; a large central laboratory serving up to 2.2 million individuals in Amsterdam and its surrounding region in the Netherlands. Individuals were enrolled based on at least one prior LDL cholesterol plasma measurement ≥99.5th percentile for their respective sex and age, based on previously reported percentiles in the LifeLines cohort in the Netherlands [14,15]. Sex- and age-specific LDL cholesterol values exceeding the 99.5th percentile, as derived from the LifeLines cohort, are detailed in Supplementary Table S1 for reference. The central laboratory database used in this study encompassed lipid profiles, predominantly requested by general practitioners (GPs), of patients in whom blood was drawn between January 2001 and January 2020. To ensure exclusion of deceased or relocated patients, this study included only those who had undergone (another, not necessarily lipid profile related) blood withdrawal at the central laboratory within a 2-year window from study initiation, spanning December 2018 to December 2020. Prior to approaching the patients, consent was sought from their GPs. Participants were enrolled between December 2020 and May 2022. The current study was approved by the Institutional Review Board of Amsterdam UMC, complied with the Declaration of Helsinki, and participants provided written informed consent.

2.2. Study procedures and definitions

The study procedures encompassed three distinct phases: phase 1 biochemical validation of hypercholesterolemia; phase 2 clinical identification of FH; and phase 3 genetic determination of FH (Fig. 1). During phase 1, patients underwent repeated blood withdrawal, including a lipid profile. Non-fasting total cholesterol, high-density lipoprotein (HDL) cholesterol, LDL cholesterol, and triglycerides were measured in lithium heparin plasma on Architect automated analyzers (Abbott) at Atalmedial Medical Diagnostic Centers. Participants who completed phase 1 were invited to complete an online questionnaire in phase 2, designed to capture details of both medical and family history, including data required for the clinical diagnosis of FH (Supplementary Table S2). Those self-reporting hypothyroidism or the use of anabolic steroids, both secondary causes of hypercholesterolemia, were excluded from the study. Finally, patients who completed phase 1 and 2 were approached for FH genetic analysis in phase 3 utilizing a customized Illumina genotyping array containing probes for 636 FH-causing variants, of which the genotyping procedures are previously described [16]. This custom-designed array previously demonstrated a sensitivity of 95% for the detection of pathogenic variants in one of the three FH genes (LDL receptor gene [*LDLR*], apolipoprotein B gene [*APOB*], and proprotein convertase subtilisin kexin type 9 gene [*PCSK9*]) [16].

Clinical FH was defined according to the Dutch Lipid Clinic Network (DLCN) criteria, utilizing the laboratory and questionnaire data gathered in phase 1 and 2. The DLCN criteria, encompassing assessment of patients' LDL cholesterol levels, family history, physical examination, and history of coronary heart disease (CHD), are endorsed by international guidelines for the clinical diagnosis of FH [6]. A score of 6 or higher, indicative of "probable" or "definite" FH, was defined as clinical FH. In the computation of the DLCN score, LDL cholesterol levels adjusted for lipid-lowering therapy (LLT) were used [15,17–24].

2.3. Statistical analysis

Categorical variables are expressed as frequencies and percentages, normally distributed variables as mean ± standard deviation (SD), and non-normally distributed data as the median and interquartile range [IQR]. Statistical analyses were performed using R version 4.0.3. Significance was determined at a threshold of $p < 0.05$ for two-sided tests.

3. Results

3.1. Descriptive characteristics

This study included participants from a central laboratory database containing lipid profiles of 590,067 individuals, of whom 15,168 had at least one prior LDL cholesterol measurement ≥99.5th percentile for their sex and age. After excluding individuals lacking blood withdrawal records within 2 years of study initiation (n = 6840) and those without consent from their GP (n = 2714), a cohort of 5614 subjects, originating from 457 different primary care practices, was approached for an additional blood withdrawal (phase 1, Fig. 1). A total of 2088 individuals (37%) consented to reassessment of their lipid levels, of whom 1273 participants (61%) further engaged in the completion of an online questionnaire (phase 2; Fig. 1). Of the 1273 participants, 170 were excluded due to (self-reported) hypothyroidism (n = 166) or use of anabolic steroids (n = 4). The characteristics of the remaining 1103 participants are shown in Table 1. The mean age was 64.2 ± 12.7 and 525 (48%) were male. History of hypertension, type 2 diabetes mellitus, and smoking was reported by 442 (40%), 120 (11%), and 96 (9%), respectively. Mean LDL cholesterol level obtained during the study was 4.0 ± 1.4 mmol/l. Among the participants, 722 (65%) were on LLT, of whom 682 (62%) used statins, 141 (13%) ezetimibe, and 22 (2%) PCSK9 inhibitors. Adjusted for LLT, mean untreated LDL cholesterol was 5.7 ± 1.9 mmol/l.

3.2. Clinical FH diagnosis and achievement of LDL cholesterol targets

Among the 1103 participants completing phase 1 and 2, 282 individuals (26%) met the clinical diagnostic criteria for FH based on a DLCN score ≥6; classifying these patients as probable (n = 195) or definite (n = 87) FH (Fig. 2). Within this group meeting the FH clinical diagnostic criteria, 41 (15%) reported a history of prior FH genetic testing, of whom 26 had been identified as carrying an FH-causing variant. Patient characteristics of the clinically diagnosed FH patients are shown in Supplementary Table S3. Among these clinically diagnosed FH patients, 233 (83%) received LLT and 33 (12%) had a history of ASCVD (secondary prevention), while the remaining 249 individuals (88%) had not experienced prior ASCVD events (primary prevention). Within the primary prevention group, 3 (1%) patients reached the European Society of Cardiology (ESC)/European Atherosclerosis Society (EAS)-recommended LDL cholesterol target of <1.8 mmol/l [25], whereas 6 (2%) patients achieved the local national guideline recommended LDL cholesterol target of <2.6 mmol/l (Fig. 3) [26]. As for the

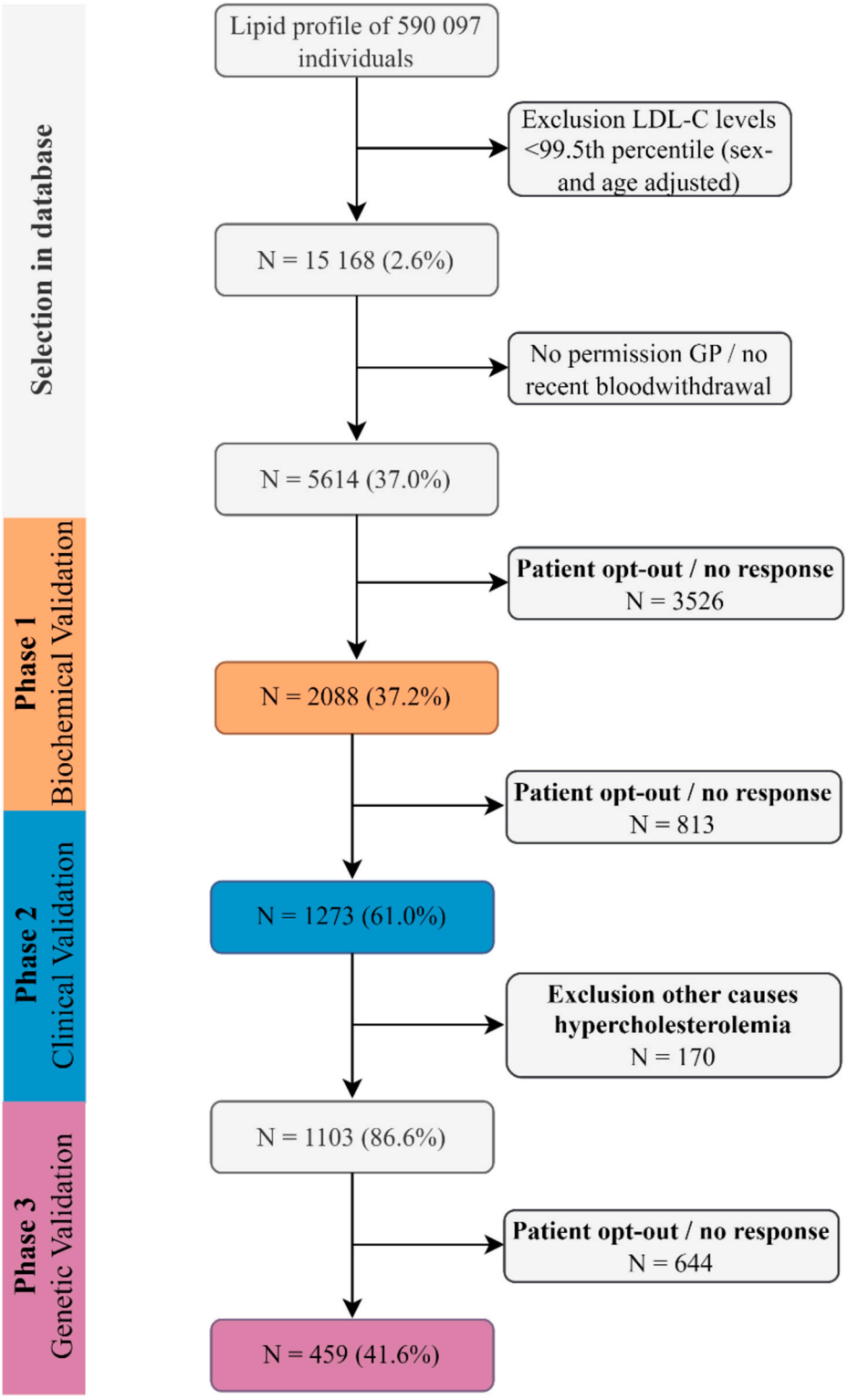

Fig. 1. Flowchart participant selection.
Initial screening identified 15,168 individuals with LDL cholesterol levels ≥99.5th percentile from a database of 590,067 lipid profiles. After excluding subjects due to outdated blood records or lack of consent, 5614 remained for the study. This cohort underwent three phases. Phase 1 involved 2088 individuals for biochemical validation of hypercholesterolemia, represented in orange. Phase 2 comprised 1273 individuals for clinical identification of FH, shown in blue. Phase 3 included 459 individuals for genetic determination of FH, depicted in purple. FH, familial hypercholesterolemia; GP, general practitioner; LDL, low-density lipoprotein. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Table 1
Participant characteristics.

Characteristic	N = 1103
Age – years	64.2 ± 12.7
Male sex	525 (48%)
LDL cholesterol – mmol/l	4.0 ± 1.4
LDL cholesterol corrected for lipid-lowering therapy – mmol/l	5.7 ± 1.8
HDL cholesterol – mmol/l	1.4 ± 0.3
Triglycerides – mmol/l	1.7 [1.3, 2.3]
Lipid-lowering therapy use	
Overall	722 (65%)
Statins	682 (62%)
Ezetimibe	141 (13%)
PCSK9	22 (2%)
History of CVD	
Overall	126 (11%)
MI	56 (5%)
CVA/TIA	77 (7%)
History of hypertension	442 (40%)
Diabetes mellitus type 2	120 (11%)
History of smoking	96 (9%)
Reported previous genetic testing for FH	74 (7%)
Presence of an FH-causing variant	37 (50%)

Displayed values are n (%), mean ± SD, or median [interquartile range]. CVA/TIA, cerebral vascular accident/transient ischemic attack; CVD, cardiovascular disease; FH, familial hypercholesterolemia; HDL, high-density lipoprotein; LDL, low-density lipoprotein; MI, myocardial infarction; PCSK9, proprotein convertase subtilisin/kexin type 9.

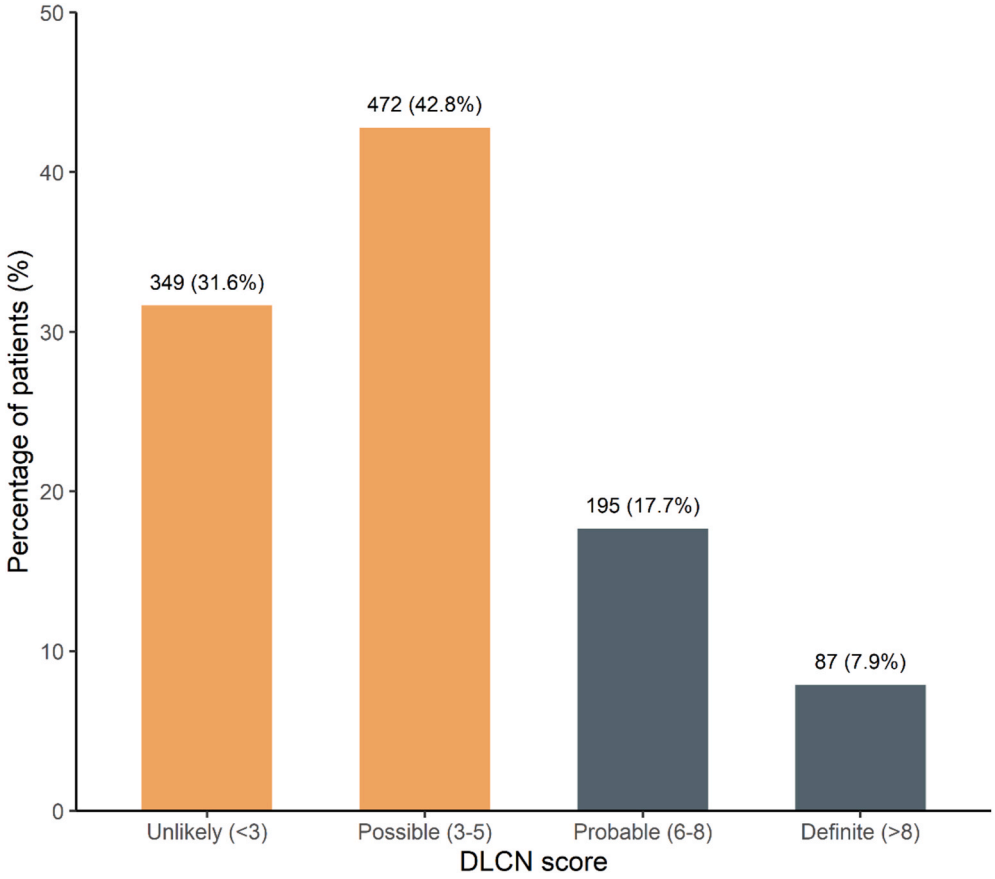

Fig. 2. Classification of participants based on DLCN scores.
Shown here is the classification of individuals who completed phase 1 (repeat blood withdrawal) and 2 (online questionnaire), based on the DLCN criteria. Based on this score, participants are classified into four groups: "Unlikely", "Possible", "Probable", or "Definite" FH. A clinical FH diagnosis is defined as a DLCN score ≥6 (grey in figure). DLCN, Dutch Lipid Clinic Network; FH, familial hypercholesterolemia.

secondary prevention group (n = 33), 1 (3%) patient reached the ESC/EAS-recommended LDL cholesterol target of <1.4 mmol/l[25], and 1 (3%) patient met the local national guideline recommended LDL cholesterol target of <1.8 mmol/l (Fig. 3) [26].

3.3. Persistent hypercholesterolemia

Among the 1103 participants who completed both phase 1 (repeated blood withdrawal) and 2 (online questionnaire; no secondary causes of

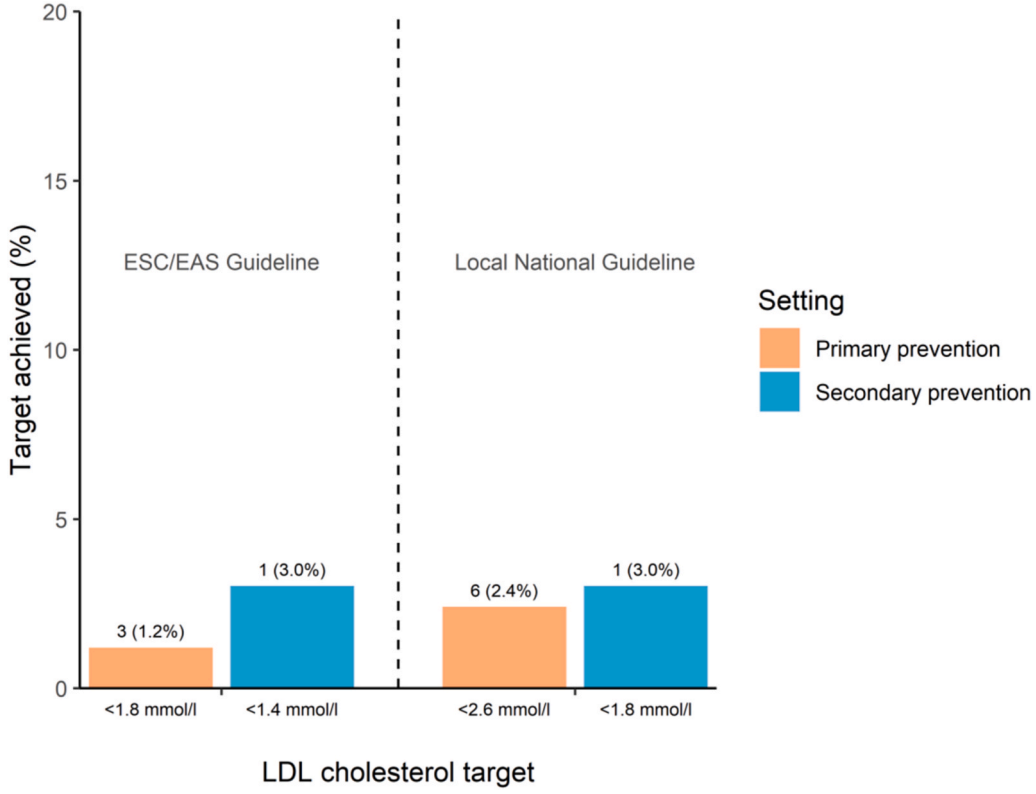

Fig. 3. Achievement LDL cholesterol targets in clinically diagnosed familial hypercholesterolemia patients.
Show here are the number of clinically diagnosed FH patients reaching the ESC/EAS-recommended LDL cholesterol targets (<1.8 mmol/l in primary prevention and <1.4 mmol/l in secondary prevention) and the local national guideline recommended LDL cholesterol targets (<2.6 mmol/l in primary prevention and <1.8 mmol/l in secondary prevention). EAS, European Atherosclerosis Society; ESC, European Society of Cardiology; LDL, low-density lipoprotein.

hypercholesterolemia), 122 (11%) had an LDL cholesterol level ≥99.5th percentile during repeated blood withdrawal. When adjusted for their individually registered LLT use, 391 (35%) of the 1103 participants had repeated LDL cholesterol measurements ≥99.5th percentile. The distribution of unadjusted LDL cholesterol levels among the 1103 participants measured during phase 2 of the study is depicted in Fig. 4.

3.4. Genetic diagnosis

Of the 1103 participants, 459 (42%) consented to genetic analysis aimed at identifying the presence of an FH-causing genetic variant (phase 3). Characteristics of this group are shown in Supplementary Table S4. Mean LDL cholesterol in this sub-cohort was 4.0 ± 1.4 mmol/l, and a total of 311 subjects (68%) received LLT. Adjusted for LLT, mean LDL cholesterol was 5.8 ± 1.9 mmol/l. Using a customized FH genotyping array in all 459 subjects, a pathogenic variant was identified in 58 (13%) participants. Of the 58 genetically confirmed FH patients, mean LDL cholesterol was 3.8 ± 1.6 mmol/l, 4 (7%) had CVD in their medical history, and 46 (79%) received LLT (Supplementary Table S5). Among the genetically diagnosed FH patients without prior CVD (n = 54), 1 patient (2%) reached the ESC/EAS-recommend LDL cholesterol <1.8 mmol/l and 10 (19%) reached the local national guideline recommended LDL cholesterol target of <2.6 mmol/l.

When restricting the selection of participants in phase 3 to those with a persistent LDL cholesterol level ≥99.5th percentile, both at first and repeat blood withdrawal (n = 155), the yield of genetic testing was 17% (26/155). When zooming in at the 282 individuals in phase 2 fulfilling the criteria for clinical FH, 118 of them proceeded with genetic validation in phase 3. Among them, 22 individuals were found to carry an FH-causing variant, resulting in a 19% (22/118) yield of genetic testing. When considering participants with both an LDL cholesterol level ≥99.5th percentile (unadjusted or adjusted in case of LLT use) combined with a clinical FH diagnosis in phase 2 (n = 95), the yield of genetic testing was 20% (19/95).

4. Discussion

Analysis of a dataset from a large central laboratory facility revealed that 1 out of every 4 individuals with a single assessment of LDL cholesterol level exceeding the 99.5th percentile (sex- and age-adjusted) meets the criteria for a clinical FH diagnosis as determined by a DLCN score ≥6 (Fig. 5). In these clinically diagnosed FH patients, 85% did not receive guideline-recommended genetic testing for FH, whereas more than 97% of them failed to meet contemporary guideline-based LDL cholesterol target levels; irrespective of whether the setting was primary or secondary prevention. In 459 individuals who consented to genetic validation, an FH-causing variant was detected in 13%. Genetic confirmation rates were 17% in participants with persistent LDL cholesterol levels ≥99.5th percentile, 19% in those clinically diagnosed with FH, and 20% in individuals meeting both these criteria. The identification of a significant number of previously undiagnosed and untreated clinical and/or genetic FH cases within a central laboratory database highlights the feasibility and clinical potential of this targeted screening strategy in identifying new FH patients and in offering opportunities to optimize LLT in this high-risk population.

Previous studies using extensive databases for FH detection have reported lower yields for clinical FH diagnoses than the current study. For example, in a cohort of over 1 million blood donors, only 0.3% met the criteria for clinical FH [13]. A recent multicenter study involving the analysis of lipid profiles in 156,082 adults in a primary-care setting led to the identification of a clinical FH diagnosis in 1% of the subjects [27]. The higher detection rate of 26% in the present study demonstrates the efficacy of using sex- and age-specific LDL cholesterol percentiles to increase the likelihood of identifying patients with clinical FH using an

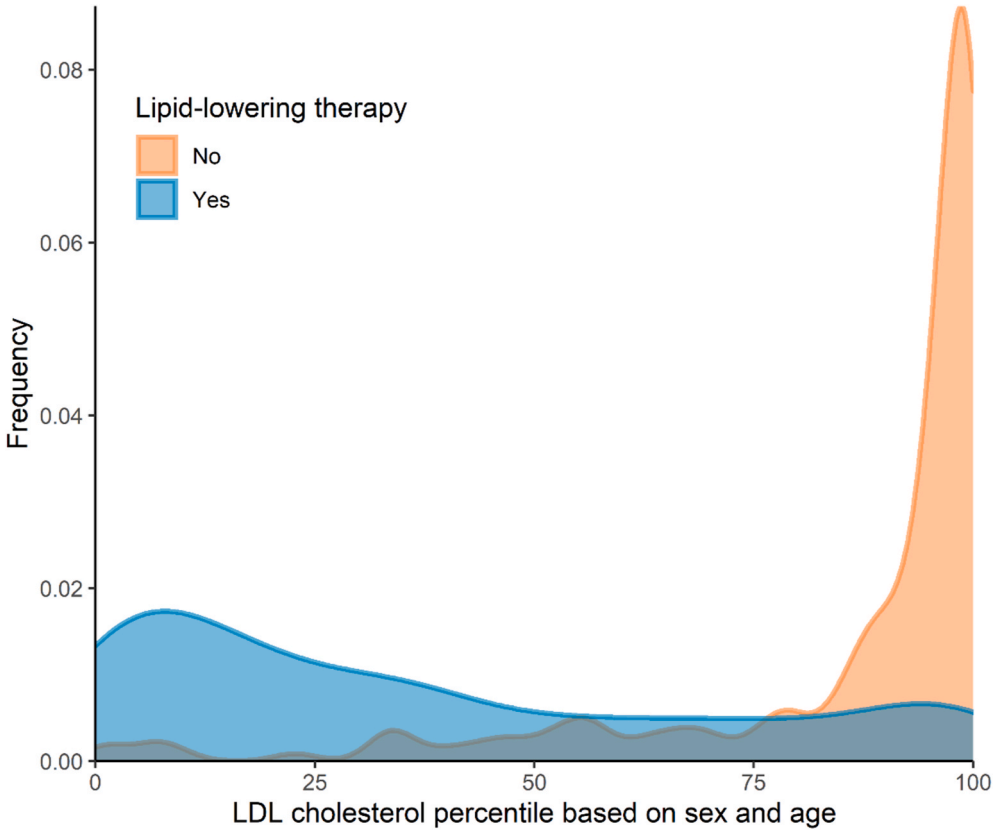

Fig. 4. LDL cholesterol distribution.
Shown here is the distribution of unadjusted LDL cholesterol levels among participants completing phase 1 (repeat blood withdrawal) and 2 (online questionnaire) (n = 1103). A distinction is made between participants receiving lipid-lowering therapy (presented in blue) and participants not receiving lipid-lowering therapy (in orange). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

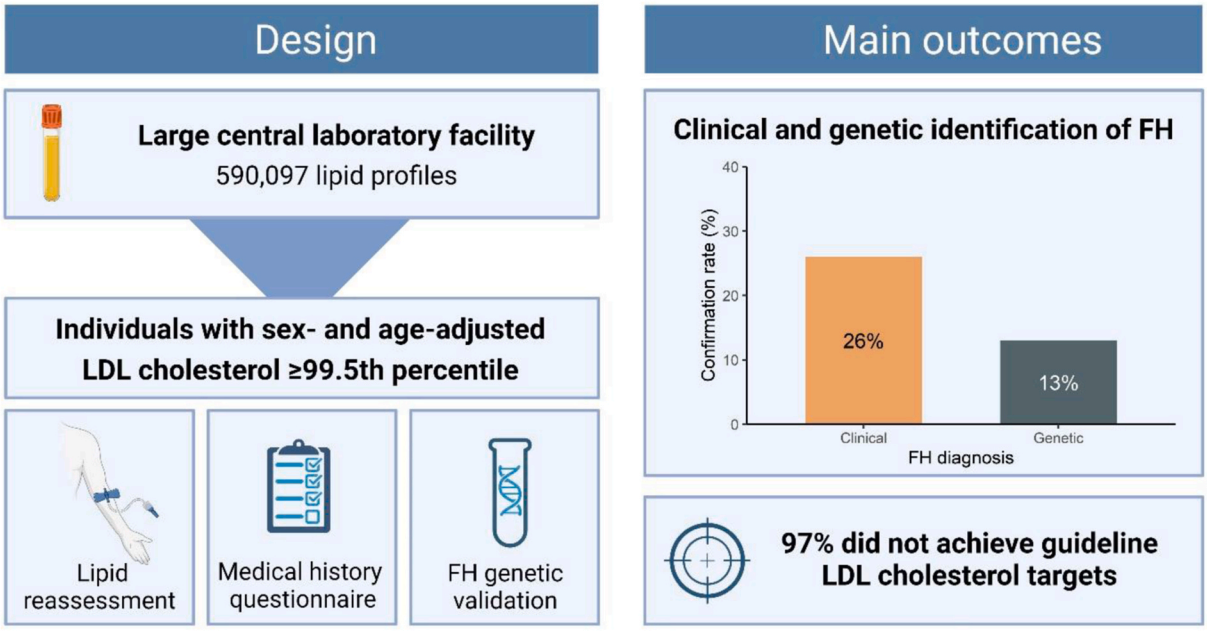

Fig. 5. Graphical abstract.
The clinical potential of an automated laboratory-based approach for the identification of clinical and genetic FH patients. FH, familial hypercholesterolemia; LDL, low-density lipoprotein.

automated, and thus low-cost, approach.

Array-based genotyping in individuals with a single LDL cholesterol measurement ≥99.5th percentile resulted in a genetic confirmation of FH in 13% of the participants. This diagnostic yield is comparable to the 14.9% genetic confirmation rate observed in patients referred for FH genetic testing by GPs and medical specialists in the Netherlands, as reported by Reeskamp et al. [28]. In their study, they demonstrated that applying the DLCN criteria resulted in a 27% diagnostic yield of FH genetic testing (with next-generation sequencing) in patients meeting the clinical criteria for FH. The current study showed a lower yield, with a 20% genetic testing yield in participants clinically diagnosed with FH and repeated LDL cholesterol levels ≥99.5th percentile. This would imply that adding clinical parameters to LDL cholesterol measurements offers limited incremental benefit for FH genetic testing. The moderate difference in diagnostic yield between the current study and that reported by Reeskamp et al., may have several explanations. First, the previous study included individuals referred to specialized lipid clinics for FH genetic testing, as opposed to the more population-wide cohort in the current study. In a referral-based population, the preselection (partly) based on clinical criteria is likely to result in a greater impact of the DLCN criteria on the yield of genetic testing. Second, in the current study, clinical parameter data were collected through questionnaires rather than being gathered at outpatient clinics by physicians, potentially leading to less accurate clinical criteria and thus less precise values. Conversely, the use of sex- and age-specific LDL cholesterol percentiles as a selection criterion in the present study, as opposed to a single absolute threshold value for LDL cholesterol used in previous studies, may have contributed to the relatively high percentage of clinical (26%) as well as genetically confirmed (13%) FH in the present study cohort.

4.1. Clinical implications

Despite longstanding efforts to increase FH detection rates, the global diagnosis rate remains less than 1%, emphasizing the need for better strategies [9]. While fully implementing all three phases of the current study in a laboratory setting may be too complex, the present study underlines the feasibility and potential of implementing a centralized strategy for FH detection by virtue of flagging patients with a single LDL cholesterol level ≥99.5th percentile for sex and age. As this process can be automated and integrated seamlessly within existing laboratory workflows and digital communication systems, the likelihood of additional costs associated with manual procedures or the need for extensive new infrastructure is minimal. Proactive collaboration between central laboratories, primary care physicians, lipid clinics and FH advocacy groups are crucial for such a strategy to be successful. The implementation of clinical decision support tools in laboratories is essential and highly endorsed by primary care physicians according to multiple survey studies [29,30]. Efficient follow-up of genetic FH screening can be facilitated in the future by broader use of high-throughput, low-cost customized FH arrays, which have been shown to result in accurate identification of FH-causing variants [16].

Despite evidence showing that genetic testing improves cardiovascular risk classification, promotes therapy initiation and adherence, and enables cascade testing in at-risk family members [7], multiple studies have confirmed underutilization of this guideline-recommended modality [1,11]. This is in line with the current study in which genetic testing was not performed in 85% of the 282 clinically diagnosed FH patients, representing a notable missed opportunity. Remarkably, among the patients newly diagnosed with an FH-causing variant in the present study, 95.2% consented to cascade testing of their relatives. Given that each index case in the Netherlands leads to the identification of an average of 8 additional FH cases [31], the approach adopted in this study holds the potential to significantly enhance the detection and subsequent management of FH cases on a broader scale.

With respect to the use of LDL cholesterol lowering treatment, guidelines have unanimously emphasized the urgency of early and potent LLT to reduce ASCVD risk in FH [25,32–39]. The present study reveals a compelling reality: 97% of clinically diagnosed FH patients fail to attain guideline-based LDL cholesterol target levels, both in the primary and secondary prevention setting. A similar pattern of undertreatment was observed among the genetically diagnosed FH patients. Collectively, these data reemphasize the need for enhanced efforts to implement diagnostic as well as stringent therapeutic guidelines in FH management. Newly identified FH patients in the current study and their GP were informed of the diagnosis, accompanied by specific treatment recommendations, aiming to facilitate the initiation and/or optimization of therapy.

4.2. Limitations

Several limitations of the present study warrant further discussion. First, not all initially selected individuals participated during all study phases, which may have introduced selection bias. Mean LDL cholesterol levels were lower in the patients that opted for genetic testing compared to the overall group. An explanation could be that the most severe cases had already been offered genetic testing before and thus opted out of additional genetic analysis. Second, the FH genotyping array used in the genetic validation phase of the study has a 95% sensitivity, resulting in a small possibility of false negative results. Hence, there may have been an underestimation of the genetic yield observed in the current study. Furthermore, the reliance on self-reported data for assessing ASCVD history and DLCN scores poses a limitation, despite measures implemented to enhance the comprehensibility of the survey. Lastly, patient-reported experience measures were not included in the current study, which may have provided a more holistic view of the effectiveness of the screening process and its implications for patients and their families.

4.3. Conclusions

Analysis of a database from a large central laboratory revealed that 1 in 4 individuals with a single LDL cholesterol measurement exceeding the 99.5th percentile (adjusted for sex and age) qualifies for a clinical FH diagnosis. Many of these individuals were not genetically tested for FH and did not attain guideline-recommended LDL cholesterol targets, underscoring the clinical potential of a laboratory based approach for the identification of new FH patients as well as for optimization of LLT in this high-risk population.

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Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: **NSN** is cofounder of Lipid Tools and reports grants from the Dutch Heart Foundation (grant number 03-007-2023-0068) and the European Atherosclerosis Society (2023). **GKH** reports research grants from the Netherlands Organization for Scientific Research (vidi 016.156.445), CardioVascular Research Initiative, European Union and the Klinkerpad fonds, institutional research support from Aegerion, Amgen, AstraZeneca, Eli Lilly, Genzyme, Ionis, Kowa, Pfizer, Regeneron, Roche, Sanofi, and The Medicines Company; speaker's bureau and consulting fees from Amgen, Aegerion, Sanofi, and Regeneron until April 2019 (fees paid to the academic institution); and part-time employment and stock holder of at Novo Nordisk A/S, Denmark since April 2019. **LFR** is cofounder of Lipid Tools and reports speakers fee from Ultragenyx, Novartis, and Daiichi Sankyo. **ESGS** has received ad-board/lecturing fees, paid to the institution, from: Amgen, Sanofi, Astra-Zeneca, Esperion, Daiichi

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CRediT authorship contribution statement

Shirin Ibrahim: wrote the manuscript, performed the analysis. **Nick S. Nurmohamed:** performed the analysis. **Erik S.G. Stroes:** Supervision. All authors discussed the results and provided critical feedback and helped shape the final manuscript.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.atherosclerosis.2024.117548.

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