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Genetic screening of children for familial hypercholesterolaemia: the VRONI study

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Abstract

Background and Aims The role of genetic testing as part of universal screening programmes for familial hypercholesterolaemia (FH) in children is not well defined. Here, a two-step approach to identify children carrying FH-causing variants was investigated.

Methods In this study from Southern Germany, paediatricians were invited to offer FH screening to all children aged 4.8–14.9 years at routine paediatric examinations. The FH screening programme began in September 2020 in Bavaria and has involved up to 480 paediatricians. It included biochemical and genetic testing using 0.2 mL of blood taken from a fingertip. In case of low-density lipoprotein cholesterol (LDL-C) serum concentration ≥3.36 mmol/L (≥130 mg/dL), FH-causing variants were determined in the same sample with a focused panel covering most frequent variants (n = 48) and sequencing of relevant genes.

Results Out of 25 431 children screened so far, 1689 children had an LDL-C ≥ 3.36 mmol/L (>130 mg/dL), which defined this concentration as the 93rd percentile. Pathogenic variants were identified by the focused panel in 157 and by next-generation sequencing in 283 children, respectively. While 17% (283/1670) of all genetically analysed children tested positive, the fraction of individuals with FH-causing variants increased across the spectrum of LDL-C serum concentrations from 4.7% (23/492) at 3.36–3.49 mmol/L (130–135 mg/dL) to 78.6% (81/103) above 5.17 mmol/L (200 mg/dL). Overall, the prevalence of FH-causing variants was high (1:90). One reason was a founder variant (n = 63) within the *LDLR* gene, found 40 times more frequent than European average. The analysis of recruitment data revealed significant ascertainment bias, with lower

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recruitment rate practices exhibiting higher prevalence. After adjustment for the bias using a generalized linear mixed model, the predicted prevalence was 1 in 163 (0.61%), which is highly consistent with large-scale genomic benchmarks as gnomAD (1:165, *n* = 622 057) and the UK Biobank (1:176, *n* = 48 741).

Conclusions The prevalence of FH determined in this study is significantly higher than previously published estimates (~1:250), highlighting the importance of this condition for public health and supporting calls for a national paediatric screening programme, given the availability of effective treatment options. For children between 5 and 15 years, biochemical screening is an effective way to select patients for genetic testing, with sequencing of candidate genes being superior to variant screening. In summary, the VRONI study demonstrates the feasibility and efficacy of a combined biochemical and genetic screening for FH in children.

Structured Graphical Abstract

Key Question

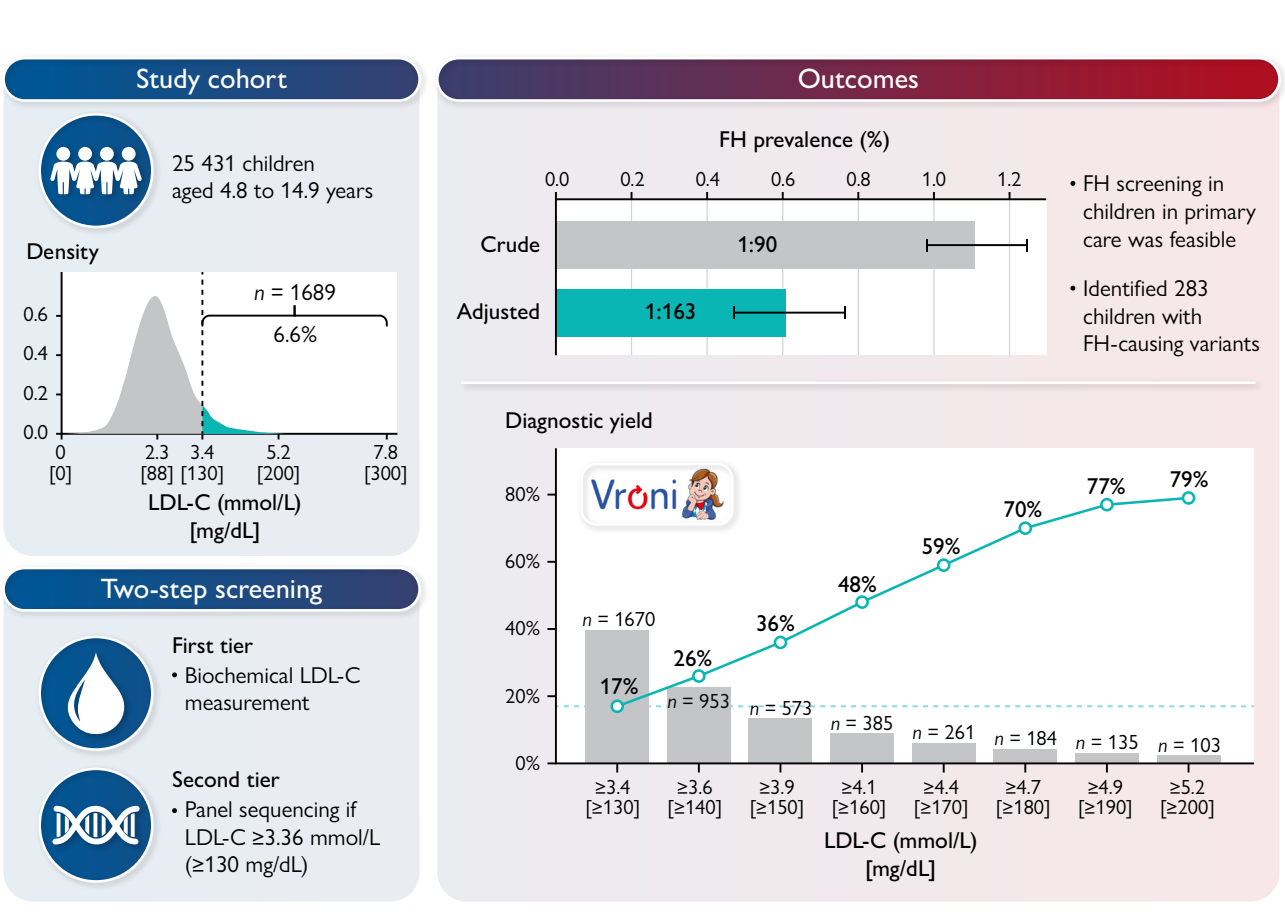
How can genetic testing for familial hypercholesterolaemia (FH) be integrated in paediatric screening at the primary care level? Does next-generation sequencing (NGS) compared to targeted panel testing improve FH detection?

Key Finding

In the VRONI registry, a total of 25,431 children were screened by primary-care paediatricians. NGS detected 283 FH cases, significantly more than a focused panel approach (n = 157). One in 163 children (0.61%) was found to carry a FH-causing variant.

Take Home Message

A structured implementation of genetic FH screening including NGS should be considered in national prevention programmes.



FH, familial hypercholesterolaemia; LDL-C, low-density lipoprotein cholesterol

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Keywords Familial hypercholesterolaemia • Genetic screening • Universal screening • Children • Next-generation sequencing • Cardiovascular disease

Introduction

Familial hypercholesterolaemia (FH) is a monogenic disorder characterized by high blood concentrations of low-density lipoprotein cholesterol (LDL-C), which results in markedly elevated risks for coronary artery disease (CAD) and premature death. FH has been reported to affect approximately 1 in 250 individuals worldwide.[1–7] In adults, cholesterol depositions in skin, cornea or tendons, or a (family) history of CAD may facilitate the diagnosis. Since clinical signs are less common and LDL-C concentrations are generally much lower in children, clinical FH scores display a poor sensitivity in childhood.[8] Therefore, the diagnosis of FH is rarely made in children and, if so, only after a severe event in the family (e.g. heart attack at a young age).[9,10] A genetic diagnosis, however, may be of particular relevance for treatment decisions in children because carriers of pathogenic variants are known to have a worse long-term prognosis even at the same LDL-C blood concentration.[2,11] Importantly, trials showing treatment benefits in children have almost exclusively included genetically proven cases.[12,13]

Variants in the *LDLR* gene account for over 90% of molecularly defined cases, while variants within *APOB* and *PCSK9* are less common, contributing approximately 5%–8% and 1% of cases, respectively.[14,15] Only in rare cases, biallelic variants in the *LDLRAP1* gene can cause a recessive inheritance pattern.[16–18] Biallelic occurrence of pathogenic or likely pathogenic *LDLR*, *APOB* or *PCSK9* variants results in a more severe clinical presentation, with earlier onset and more pronounced cardiovascular complications.[19]

There is no consensus on how to arrive at an early (genetic) diagnosis.[20–22] Screening of children may be carried out either opportunistically, i.e. after a clinical event in the family, or in families with an established molecular diagnosis of FH. Cascade screening intends to identify all affected first- and second-degree relatives of a variant carrier.[23] However, the FH detection rate varies substantially between countries, with high identification rates only observed in regions with long-established, well-organized cascade screening programmes, such as in Norway and the Netherlands. Where cascade screening is incomplete or lacking, universal paediatric screening provides a more effective alternative, as demonstrated in Slovenia and, more recently, Croatia.[10] A universal screening is therefore recommended by various academic societies, such as the European Atherosclerosis Society (EAS), the National Lipid Association (NLA) and the American Academy of Pediatrics (AAP), and is listed as a priority action by the European Union within its 2021 call for a 'Public Health Genomics' approach to improve the early detection of rare and genetic diseases. However, only Slovenia and Slovakia have established a national programme.[22] Hopefully the high sensitivity and increased affordability of next-generation sequencing (NGS) is likely to have profound impact on the decision to adopt universal FH testing in many healthcare systems.

The VRONI study is a population-oriented FH screening programme in Germany at the primary care level. Participating paediatricians voluntarily enrol children aged 4.8–14.9 years for a combined approach including LDL-C concentration measurements and, as a second-tier test, NGS genetic analysis in hypercholesterolaemic children.

The overarching goal of VRONI is to serve as a pilot for future universal FH screening programmes. Our first major aim was to show that a combined biochemical and genetic approach allows to identify relevant numbers of affected children that should receive medical treatment according to current guidelines.[24] Our second major aim was to show that such programme can be implemented within established healthcare structures at the primary care level and does not require cost-intensive dedicated visits.[25] In addition to identifying children with FH, we here present the genetic results from the VRONI project, focusing on pathogenic or likely pathogenic variants and the diagnostic yield of genetic testing.

Materials and methods

Study cohort

The VRONI study is part of DigiMed Bayern, a pilot project for predictive, preventive, personalized and participatory medicine (P4) in Germany, with the detailed study procedures having been published previously.[26] A flow chart providing a more detailed overview is also available in the Supplementary material. VRONI is a population-oriented screening programme for FH in children, initially launched in Bavaria. Designed as a proof-of-concept study, it is intended to later serve as a best-practice example for a nationwide universal screening programme. In brief, all board-certified paediatricians in Bavaria ($n = 1309$) were invited via the Professional Association of Paediatricians (BVKJ). Participation was voluntary. Paediatricians were encouraged to offer screening to all children in the context of the publicly recommended and health insurance-funded recurring routine examinations U9 (58–66-month olds), U10 (7–8-year olds), U11 (9–10-year olds) and J1 (12–14-year olds), irrespective of family history or perceived risk, with the aim of approximating a population-based sample. The participation rate of the U9 is 98% in Germany.[27] Enrolment during other patient visits for those aged 4.8–14.9 years was also permitted.

Participation of the children was voluntary as well. While the voluntary participation of both paediatricians and patients allows to study the utilization of an FH screening in a real-world setting of patient care, we are aware that by design our study did not reach all children at risk. The screening programme began in September 2020, with 480 paediatricians participating until October 2024, which equates to 36% of all practising paediatricians in Bavaria. A 0.2 mL EDTA capillary blood sample was taken from each child and sent to the study centre at the German Heart Centre Munich (DHM) via postal service for the

initial determination of blood LDL-C concentration. Children with an LDL-C ≥ 3.36 mmol/L (≥130 mg/dL) were planned for genetic analyses. This threshold corresponds approximately to the 95th percentile of the LDL-C concentration in this age group, clinically defines hypercholesterolaemia and aligns with the recommended concentration for considering statin therapy in genetically confirmed FH cases. Clinical chemistry analyses on plasma were performed centrally at the German Heart Centre under pseudonyms and data was then transferred internally to the VRONI database for secure storage and analysis. In children with hypercholesterolaemia, a second venous blood sample was obtained for extended lipid profiling including total cholesterol, LDL-C, high-density lipoprotein cholesterol, triglycerides, and lipoprotein(a). Fasting was not mandatory, and triglyceride values were therefore interpreted with caution. At study inclusion, standardized questionnaires captured anthropometric data (weight, height, waist circumference), number of siblings, parental birth years, and family history of hypercholesterolaemia or premature cardiovascular disease. Physical stigmata such as tendon xanthomas or corneal arcus were not systematically assessed within the study protocol. Family history and anthropometric characteristics are summarized in *Table 1*. Informed consent was obtained prior to study participation in accordance with local regulations. Due to the sensitive nature of personalized data, the VRONI study focused on strict data protection by separating identifying information from research data, using randomly generated pseudonyms and a two-stage pseudonymization process in full compliance with German data protection and security regulations. All analyses are performed exclusively on pseudonymized data. The modular VRONI database securely integrates medical, genetic, and analytical data and is hosted in a protected container environment on university servers at the German Heart Centre, a university hospital of the Technical University of Munich. The study was approved by the ethics committee of the Technical University of Munich and was conducted in accordance with the requirements of the Helsinki Declaration of 1975, as revised in 2013.

Genetic analyses

Molecular genetic analyses of two-stage pseudonymized samples were conducted at the Institute of Human Genetics (IHG) at the Technical University of Munich (TUM) and the Institute of Neurogenomics (ING) at Helmholtz Munich (Helmholtz Zentrum München, Deutsches Forschungszentrum für Gesundheit und Umwelt, HMGU). Genetic screening was performed using an NGS panel specifically designed for FH on a NovaSeq 6000 (Illumina, CA, USA) as previously described.[26] The entire genomic regions of *LDLR*, *APOB* and *PCSK9* were sequenced, including promoter and intronic regions, but excluding repetitive intronic regions. Moreover, the targeted NGS panel (TWIST Bioscience, CA, USA) was customized for the exonic regions of 23 genes involved in cholesterol metabolism (see Supplementary data online, *Table S1*). Sequencing reads were mapped to human genome build GRCh37/hg19. A genetic variant with an allele frequency of <0.1% was classified as pathogenic, if it was either listed in ClinVar as 'likely_pathogenic' or 'pathogenic', or if it was assessed as 'likely_pathogenic' or 'pathogenic' according to the published American College of Medical Genetics and Genomics (ACMG) criteria.[28] Nomenclature for the description of sequence variants follows the recommendations of the Human Genome Variation Society and refers to transcripts NM_000527.5 and NM_000384.3 of *LDLR* and *APOB*, respectively.[29] Carriers of 'likely_pathogenic' or 'pathogenic' variants in *LDLR* or *APOB* were defined to have 'monogenic FH', all other cases to have 'non-monogenic hypercholesterolaemia'. A scientific genetic report detailing the results of panel sequencing was issued via the VRONI study centre, which de-pseudonymized the sample ID and forwarded the report to the referring paediatrician, who then communicated the results to the family. All positive test results were genetically validated via a second venous blood sample.

Since there was no other large-scale sequencing data of children (with LDL-C concentrations) available to us, we used gnomAD as an external reference for the allele frequencies of the identified variants. We acknowledge that gnomAD covers an adult population but do not anticipate any relevant limitation in this regard since FH variants are germline and their population allele frequency is largely independent of age. Moreover, gnomAD is not a random population sample that may be depleted of pathogenic variants due to the composition of the cohort ('healthy participants'). Therefore, we limited our comparisons to the closest available ancestry subset (non-Finnish European) and interpreted the results cautiously, bearing in mind the differences in ancestry structure and variant calling/annotation pipelines between gnomAD and our screening cohort. Estimation of FH prevalence in gnomAD variants was processed using the same filtering and classification procedure as in the VRONI genetic analysis. Individuals with at least one FH-qualifying variant were counted as carriers and used for FH prevalence estimation.

Founder variant analysis

DNA samples from suspected founder variant carriers were genotyped using GSA array and processed in PLINK (.ped/.map) format,[30] aligned to the GRCh37 reference,[31] and merged into a single dataset. The merged genotype dataset was then phased using Beagle[32] to resolve haplotypes across markers. The phased haplotypes were extracted from each dataset and combined. A consensus variant-bearing haplotype was inferred by aligning all carriers' haplotypes and taking the allele shared by all carriers at each single nucleotide polymorphism (SNP). SNP genotypes flanking the variant were aligned across individuals, and contiguous blocks of identical alleles among carriers were identified as shared ancestral segments. These shared segments define the core haplotype around the suspected variant. Subsequently, the set of conserved segment lengths (from all carriers) was used to estimate the time that elapsed since the founder variant. We applied the Gamma-distribution-based method of Gandolfo *et al.* via their online R Shiny mutation-dating tool.[33,34] In this approach, the total ancestral length (sum of the left and right arm lengths) is modelled as a Gamma $(2, \tau)$ random variable, where τ is the variant age in generations.[33] The input segment lengths were used to compute the maximum-likelihood estimate of τ with small-sample bias correction and the associated 95% confidence intervals (CI). The haplotype alignments indicated that some carriers share more recent common ancestors than the most recent common ancestor of the entire cohort. Therefore, correlated genealogy

Table 1 Baseline characteristics of the VRONI cohort overall, and of the subgroups of children undergoing genetic analysis, further stratified into those with familial hypercholesterolaemia and non-monogenic hypercholesterolaemia

Characteristics	Overall Cohort	Genetic Subcohort	FH	NMH
Total sample size, *n*	25 431	1670	283	1387
Ethnicity	European 93.4%; African 1.5%; Arab 2.6%; Asian 2.9%; Other 2.7%	European 92.7%; African 2.1%; Arab 2.3%; Asian 2.6%; Other 3.3%	European 93.1%; African 2.5%; Arab 1.0%; Asian 2.0%; Other 3.4%	European 92.6%; African 2.0%; Arab 2.6%; Asian 2.8%; Other 3.3%
Age (years)	9.1 [6.5–12.1]	9.8 [7.4–12.4]	9.7 [7.5–12.6]	9.8 [7.3–12.4]
Female sex (%)	48.4% (12 307/25 431)	53.8% (899/1670)	49.1% (139/283)	54.8% (760/1387)
Weight (kg)	32.3 [23.0–47.0]	36.0 [25.0–49.3]	35.7 [24.8–46.6]	36.3 [25.0–50.0]
Height (cm)	137.5 [121.0–154.2]	140.0 [125.0–154.0]	143.0 [124.0–155.0]	139.4 [125.0–154.0]
BMI (kg/m^2)	16.9 [15.2–19.9]	17.9 [15.6–21.5]	17.3 [15.6–19.5]	18.1 [15.6–21.7]
Obesity (%)	5.8% (1083/18 620)	9.2% (117/1277)	5.0% (11/221)	10.0% (106/1056)
Positive family history (%)	24.2% (6165/25 431)	29.4% (491/1670)	35.7% (101/283)	28.1% (390/1387)
Lipid profile, mmol/L (mg/dL)				
Total cholesterol	4.27 [3.83–4.78] 165.0 [148.0–185.0]	5.82 [5.51–6.26] 225.0 [213.0–242.0]	6.46 [5.86–7.20] 250.0 [226.8–278.2]	5.74 [5.46–6.13] 222.0 [211.0–237.0]
LDL-cholesterol	2.28 [1.91–2.72] 88.1 [74.0–105.0]	3.67 [3.47–4.06] 142.0 [134.0–157.0]	4.47 [3.85–5.29] 173.0 [149.0–204.5]	3.62 [3.47–3.88] 140.0 [134.0–150.0]
HDL-cholesterol	1.44 [1.24–1.66] 55.7 [47.8–64.1]	1.50 [1.29–1.72] 58.1 [49.8–66.5]	1.40 [1.21–1.58] 54.0 [46.9–61.0]	1.52 [1.31–1.75] 58.9 [50.5–67.5]
Non-HDL-cholesterol	2.81 [2.39–3.29] 108.6 [92.6–127.1]	4.28 [3.97–4.75] 165.5 [153.7–183.6]	5.00 [4.44–5.83] 193.5 [171.8–225.6]	4.19 [3.93–4.56] 162.1 [151.8–176.4]

Data are presented as *n*, %, or median [interquartile range], with denominators indicated where percentages are shown. Obesity defined as BMI ≥97th percentile for age and sex according to German KiGGS reference data.
BMI, body mass index; FH, familial hypercholesterolaemia; LDL, low-density lipoprotein; HDL, high-density lipoprotein; NMH, non-monogenic hypercholesterolaemia.

implementation of the Gamma method was used to estimate the pairwise correlation among segment lengths directly from the data to adjust the effective sample size. The final age estimate is reported in generations and converted to years, with a 20-year time period assumed per generation.

Statistical analyses

Data visualization and statistical analyses were performed using Python version 3.9 and R version 4.4.1. Wilcoxon tests were used to compare numerical variables between two groups. Pearson's correlation tests were used to measure relationships between two continuous variables. Chi-squared tests were used to compare two categorical variables. *P*-values in all figures are annotated as follows: $P > .05$ (NS), $P \leq .05$ (*), $P \leq .01$ (**), $P \leq .001$ (***) or $P \leq .0001$ (****). For all descriptive figures illustrating prevalence or family history by recruitment bins, error bars represent ± 1 standard error of the proportion to visualize measurement precision while maintaining clarity of the global trend.

For ascertainment bias evaluation, meta-regression was performed using a binomial generalized linear model (GLM) via the Python *statsmodels* library. The probability of identifying a carrier was modelled as a function of the log-transformed recruitment rate (patients enrolled by practice per active months). Overall, three strategies were applied to estimate prevalence while adjusting for identified bias. Initially, a conservative, exclusion-based prevalence estimate was calculated by restricting the cohort to practices that had achieved clinical saturation (defined as a recruitment intensity of ≥10 children per month). Then, to leverage the full dataset, a standard random-effects meta-analysis was conducted. Between-study variance (τ^2) was estimated via restricted maximum likelihood (REML) optimization, implemented in Python using *SciPy*. Thirdly, to address the potential disproportionate weighting of practices with low recruitment, a one-stage individual participant data (IPD) meta-analysis was performed using a generalized linear mixed model (GLMM) with a logit link function via the R package *lme4*. The model was specified as follows:

$$\log it(P(Y_{ij} = 1)) = \beta 0 + \beta 1 \ \log(Recruitment\ Rate_i) + u_i$$

where Y_{ij} is the carrier status of child *j* in practice *i*, $\beta 1$ represents the fixed effect of the log-transformed recruitment intensity (cases enrolled by practice per active months), and u_i is the random intercept for practice *i* used to account for within-centre clustering. The prevalence was estimated by calculating the marginal predicted probability at a standardized recruitment rate of 10 patients per month representing. While empirical observations indicated an initial plateauing of prevalence between 5 and 10 patients per month, this specific threshold of 10 was selected by identifying the mathematical saturation point where the first derivative of the model's prediction curve fell below 0.0005, ensuring the estimate is taken from a stable region where selective recruitment bias is minimized.

For GLMM estimate, 95% CIs were derived using the Profile Likelihood method via the *confint* function. The Clopper-Pearson (exact) method was used to calculate 95% CI for the unadjusted and exclusion-based counts.

To determine the effect of a founder variant (*LDLR* c.798T > A, p.Asp266Glu) on prevalence, the carriers were removed from the cohort, and the GLMM was re-fitted to the remaining population to estimate the predicted prevalence at recruitment intensity of 10. To account for potential familial clustering, we identified siblings within the cohort. A sensitivity analysis was performed by restricting the dataset to independent subjects, excluding five siblings from the primary prevalence calculation.

Results

Study population

From September 2020 till October 2024, a total of 25 431 children were enrolled in the VRONI screening programme for FH (51.6% boys and 48.4% girls). The median age at enrolment was 9.1 years with peaks observed in the age ranges of recommended routine examinations (see Supplementary data online, *Figure S1*). LDL-C concentrations followed a near normal distribution with a median of 2.28 mmol/L (88.1 mg/dL) and a range of 0.29–8.46 mmol/L (11.3–327.0 mg/dL) (*Structured Graphical Abstract*). The predefined LDL-C threshold for FH gene sequencing of 3.36 mmol/L (130 mg/dL) was exceeded by 1689 children (6.6%; 53.8% girls; incomplete or withheld genetic consent in 19 cases) with a median age of 9.8 years. A detailed breakdown of the demographic data for the various subgroups can be found in *Table 1*. The median LDL-C concentrations differed only slightly between age groups (see Supplementary data online, *Table S2*).

Diagnostic rate

Using a targeted approach previously described in Wald *et al.* analysing only the 48 most common FH-causing variants (46 in the *LDLR*), we identified 157 FH cases.[3] NGS documented 126 additional FH cases with pathogenic or likely pathogenic (P/LP) variants for a total of 283 cases out of 1670 hypercholesterolaemic children undergoing genetic testing. Thus, the diagnostic yield by sequencing was 78% higher than that of the targeted approach (157/1670 vs 283/1670) (*Figure 1A*). All children in whom relevant variants were found were heterozygous carriers of their respective variants, no individuals with biallelic variants were detected. Overall, a total of 17% of genetically tested hypercholesterolaemic children or 1.1% of the total VRONI cohort were found to carry an FH variant. All genetic findings were confirmed in a second independent sample. Hypercholesterolaemia without a P/LP variant in the screened genes is hereafter referred to as non-monogenic hypercholesterolaemia (NMH).

Whether screening for FH during childhood is more effective than screening during adulthood has not yet been answered. Thus, we explored the diagnostic yield of the two-step screening approach for FH-causing variants in *LDLR*, *APOB*, and *PCSK9* in an adult population-based cohort (Khera *et al.*)[2] vs our paediatric cohort. As LDL-C concentration varies with age, absolute cut-offs are not directly comparable, therefore the 93rd age-specific percentile for each group was used. Among adults above the 93rd percentile (LDL-C ≥ 4.92 mmol/L or ≥190 mg/dL), 1.7% (24/1386) were reported to be carriers of an FH-causing variant. In our study, 17% of children exceeding the 93rd percentile (LDL-C ≥ 3.36 mmol/L or ≥130 mg/dL) were found to carry an FH variant, indicating nearly a 10-fold higher diagnostic yield in a paediatric setting (*Figure 2*).

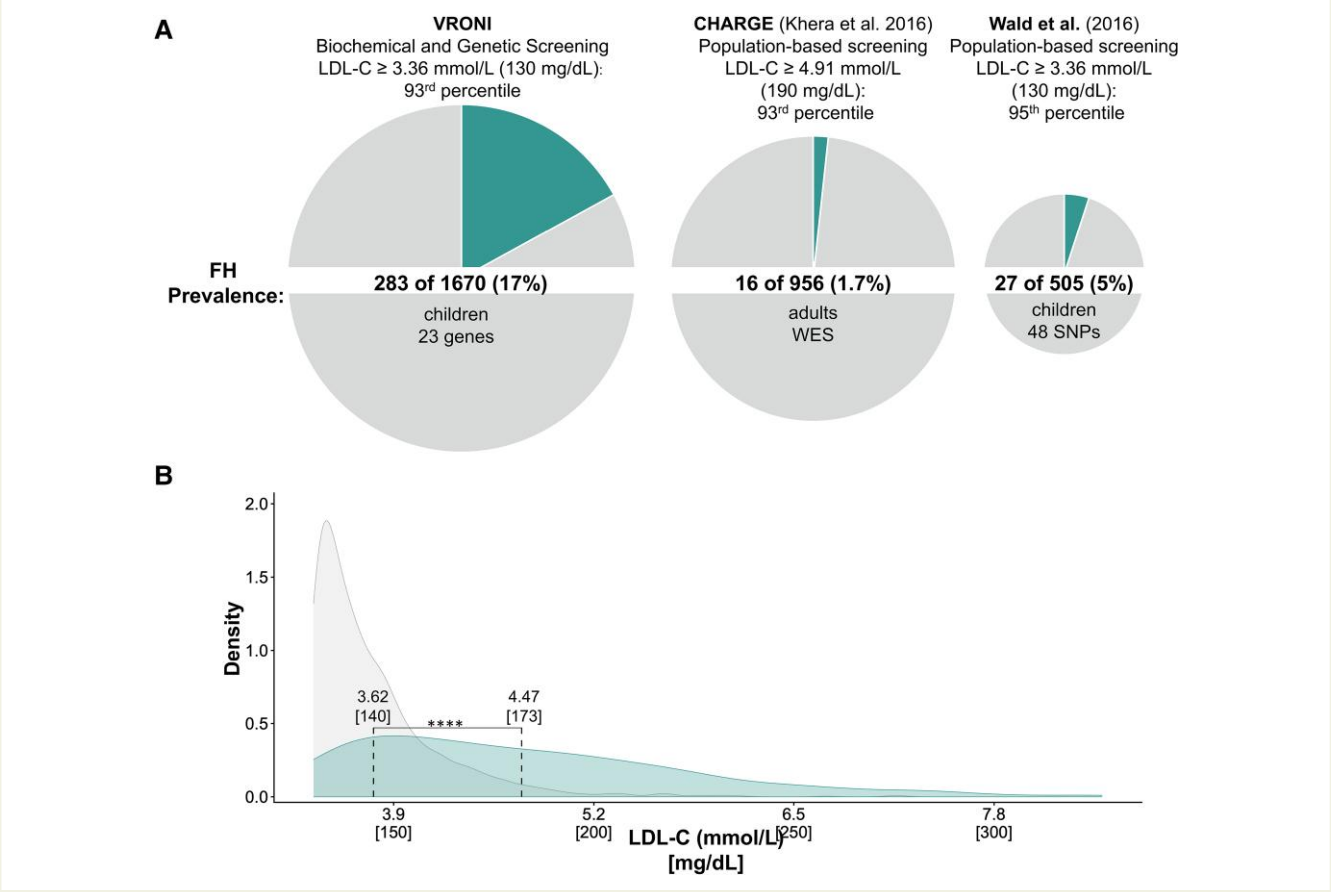

Figure 1 (*A*) Diagnostic yield of FH gene sequencing in severe hypercholesterolaemia across two paediatric and one adult cohort. The proportion of FH cases is indicated by the green segment of each circle, and circle size reflects relative cohort size. (*B*) LDL-C distribution by genetic status in the genotyped sub-cohort (N = 1670; LDL-C ≥ 3.36 mmol/L [≥130 mg/dL]), shown as a density plot: non-monogenic hypercholesterolaemia (NMH; light grey area, n = 1387) and genetically defined FH variant carriers (teal area, n = 283). Mean LDL-C concentrations are indicated by dashed vertical lines (3.62 mmol/L [140 mg/dL] for NMH and 4.47 mmol/L [173 mg/dL] for FH). Statistical significance is indicated by asterisks (****, $P < .0001$)

Prevalence of familial hypercholesterolaemia

The 283 children with FH variants correspond to an FH prevalence of 1 in 90 (1.11%, 95% CI: 0.98–1.25%), which was 2.8 times higher than reported in earlier studies (1:250).[1–3,11,35] Statistical analysis revealed an inverse relationship between practice recruitment rate and observed FH prevalence (meta regression β=−0.56, $P < .0001$), confirming the presence of systematic ascertainment bias (see Supplementary data online, *Figure S2A*). In practices that recruited fewer than 1 child per month the observed prevalence was 1 in 22, likely reflecting a selective screening approach focused on children with a known family history or symptomatic hypercholesterolaemia (See Supplementary data online, *Figure S2 B, C*).

Children screened in practices with a low recruitment rate (<1 patient per month) exhibited a significantly higher median LDL-C concentration of 2.55 mmol/L (98.6 mg/dL), IQR 2.09–3.10 mmol/L (80.8–120 mg/dL) compared to those from practices with higher recruitment rates (>20 patients per month) with a median of 2.22 mmol/L (85.8 mg/dL), IQR 1.87–2.61 mmol/L (72.3–101 mg/dL). Furthermore, practices with lower recruitment rate reported a higher frequency of positive family history of premature cardiovascular events (45.3% in low-recruiting vs 23.9% in high-recruiting practices; $P < .0001$) and hypercholesterolaemia (74.6% in low-recruiting vs 42.2% in high-recruiting practices; $P < .0001$). However, the proportion of children enrolled in low-recruiting practices was only 5.6% (1434/25 432).

To correct for the bias, we have tested several different approaches. Initial naive filtering approach (excluding all practices with a recruiting rate of <10 cases per month) yielded a prevalence of 1 in 165 but required the exclusion of 15 393 participants (60% of the cohort), significantly reducing the statistical power and generalisability of the findings. To maximize the utility of the data, we subsequently applied a REML, which yielded an FH frequency estimate of 1 in 125. However, this estimate was still inflated because substantial heterogeneity between practices ($\tau^2 = 0.55$) resulted in the excessive relative weighting of cohorts with a low recruitment rate, thereby moving the pooled estimate upwards and away from the true population mean. Finally, to utilize the full dataset while correcting for continuous bias across the recruitment spectrum, we used a one-stage IPD GLMM. To derive an unbiased population estimate, we needed to identify a point of clinical saturation—the recruitment intensity at which selective testing ceases and the

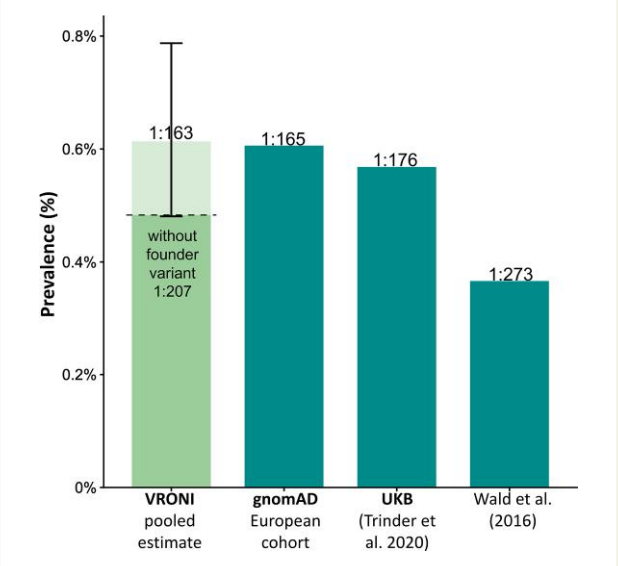

Figure 2 Prevalence of FH in VRONI and comparable cohorts. The first bar (light green) represents the bias-adjusted VRONI prevalence estimate of 1 in 163 (0.61%; 95% CI: 0.47%–0.77%), calculated using individual participant data in a generalized linear mixed model at clinical saturation (recruitment rate >10). The horizontal dashed line indicates the adjusted prevalence after excluding the *LDLR* p.Asp266Glu founder variant (1 in 207; 0.48%; 95% CI: 0.35%–0.63%). Reference bars (dark green) show prevalence in the gnomAD European cohort (1:165), UK Biobank (1:176), and the study cohort from Wald et al.[3] (1:273)

estimates approach true universal screening. While empirical data indicated an initial plateauing of prevalence in practices recruiting 5–10 patients per month, the frequency of positive family history required higher recruitment volumes to reach the true population estimates. Additional evaluation of the model's prediction curve demonstrated that the tangent slope approached zero at a rate of 10 patients per active month. After adjusting for the log-transformed recruitment rate and accounting for centre-level clustering as a random intercept, the predicted prevalence at universal screening saturation (rate = 10) was 1 in 163 (0.61%; 95% CI: 0.47%–0.77%).

This bias-corrected estimate is highly consistent with international genomic benchmarks, aligning closely with our analysis of the gnomAD cohort (1 in 165; $n = 622\,057$) using the same diagnostic criteria, and reported data from the UK Biobank (1 in 176; $n = 48\,741$).[6] Sensitivity analyses excluding five siblings (1 in 165; 0.61%; 95% CI: 0.47–0.76%) or the identified founder variant (1 in 207; 0.48%; 95% CI: 0.35–0.63%) further confirmed the robustness of the GLMM-adjusted estimate (see Supplementary data online, *Figure S3*).[6]

Phenotypic effects of FH-causing variants

LDL-C concentrations were significantly higher in genetically confirmed FH cases (median LDL-C 4.47 mmol/L (173 mg/dL) compared to NMH cases (median LDL-C 3.62 mmol/L (140 mg/dL)), with a difference of 0.85 [0.67–0.87] mmol/L (33 mg/dL [25.9–33.6 mmol/L]; $P \leq .0001$, Wilcoxon rank sum test) (*Figure 1B*). With increasing LDL-C concentration, the proportion of children carrying FH-causing variants steadily increased and reached more than 50% at 4.3 mmol/L (165 mg/dL). Correlating LDL-C bins with the proportion of identified FH cases showed similar data. The diagnostic yield of FH variants started with 4.7% (23/492) at 3.36–3.49 mmol/L (130–135 mg/dL) and increased to 78.6% (81/103) in children with LDL-C concentrations ≥5.17 mmol/L (≥200 mg/dL). This increase in diagnostic yield at higher LDL-C thresholds was accompanied by a proportional decline in sensitivity, reflecting the inherent trade-off between the efficiency of detection and the number of missed FH cases. A receiver operating characteristic (ROC) curve analysis (see Supplementary data online, *Figure S4*), restricted to the genetically analysed subpopulation (i.e. LDL-C concentrations ≥3.36 mmol/L or ≥130 mg/dL), suggested an optimal threshold of 4.03 mmol/L (156 mg/dL) for sensitivity and specificity (Youden's index). However, this analysis is potentially affected by verification bias and may therefore be misleading, as genetic status data were unavailable for children with LDL-C < 3.36 mmol/L (<130 mg/dL). Furthermore, a threshold of 4.03 mmol/L (156 mg/dL) would miss 32% of cases (91/283) with FH-causing variants. When optimized for a sensitivity of 95%, the corresponding threshold was 3.47 mmol/L (134 mg/dL), and for 90% sensitivity, it was 3.54 mmol/L (137 mg/dL).

Repeated measurements of elevated LDL-C concentrations may serve as a clinical indicator for FH in children. Thus, we obtained a second blood sample in 1190 children with initial LDL-C measurements ≥3.36 mmol/L or ≥130 mg/dL who underwent genetic assessment. Of these, 42% (506/1190) had LDL-C concentrations below the predefined threshold of 3.36 mmol/L (130 mg/dL). Median time between first and second blood sample were 206 [161–281] days. Exclusion of children below the LDL-C threshold in the second sample would improve the diagnostic yield to 29% (197/684), but on the other hand, would miss 31 out of the entire group of 228 of FH variant carriers in 1190 samples (14%).'

LDL-C concentrations in genetically confirmed FH cases ranged from 3.36 to 8.46 mmol/L (130–327 mg/dL). Thus, LDL-C concentrations in carriers of FH-causing variants were on average 2.43 [2.23–2.48] mmol/L, or 94 mg/dL, higher than the mean of the overall VRONI cohort (2.35 [2.34–2.36] mmol/L; 90.8 [90–91] mg/dL) (*Figure 3B*).

This effect was most pronounced in truncating (stop, frameshift, splice) variant carriers with a mean increase of 3.1 mmol/L (95% CI 2.79–3.50), i.e. 122 mg/dL (95% CI 108–135 mg/dL). Consequently, 70% (26/37) of carriers of truncating variants presented with LDL-C concentrations ≥4.91 mmol/L (≥190 mg/dL). In contrast, only 32% (77/240) of missense variant carriers showed such a high LDL-C concentration. The two most common variants in our study, *LDLR* p.Asp266Glu ($n = 63$) and *APOB* p.Arg3527Gln ($n = 46$), showed similar effects, with median LDL-C increases of 2.0 mmol/L (77 mg/dL) and 2.1 mmol/L (81 mg/dL), respectively. However, even in patients carrying the same variant, here exemplified by *LDLR* p.Asp266Glu, a broad range of LDL-C concentrations was observed (3.36 to 6.54 mmol/L or 130 to 253 mg/dL). Notably, these variations in LDL-C concentration could not be attributed to factors such as gender or body mass index.

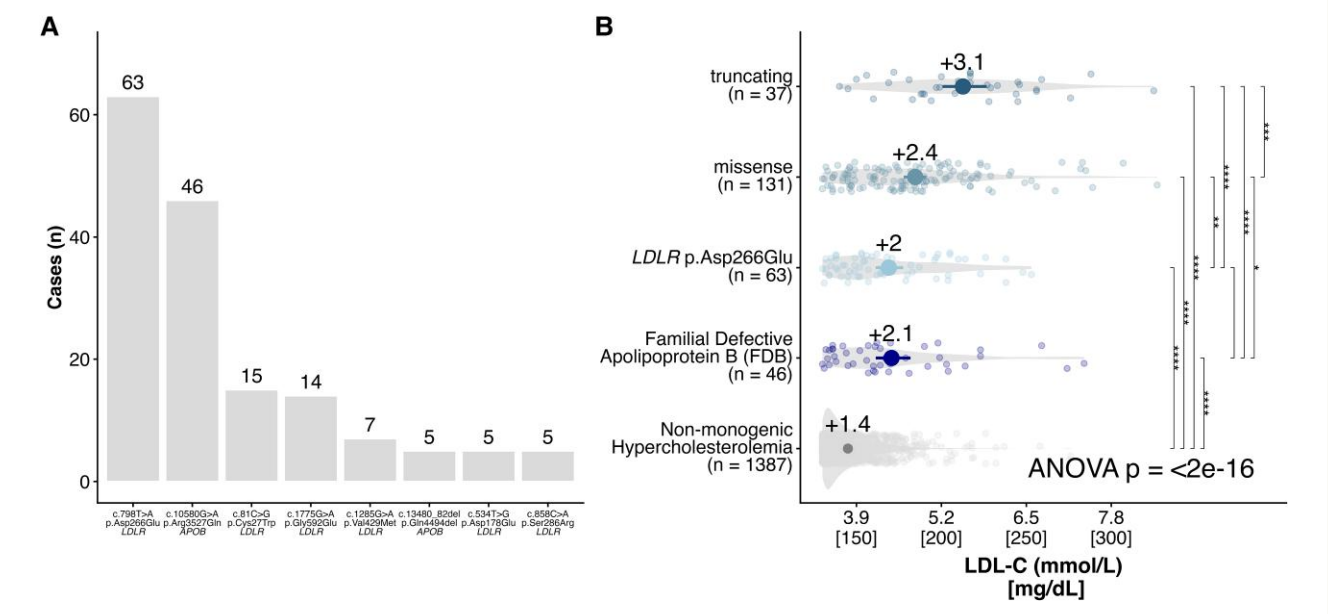

Figure 3 Genetic spectrum of FH-causing variants and their phenotypic impact. (*A*) Frequency distribution of the most prevalent FH-causing variants identified in the VRONI cohort (n ≥ 5). (*B*) Impact of different FH-causing variants on LDL-C concentration (n = 1670). Small points represent individual cases, while large circles and horizontal error bars denote the mean and 95% confidence intervals for each variant category. Numbers above each group indicate the mean LDL-C increase (in mmol/L) relative to the overall VRONI cohort median of 2.35 mmol/L (91 mg/dL). Statistical significance is indicated by asterisks based on pairwise two-sided t-tests with Benjamini-Hochberg multiple-testing correction (*P < .05, **P < .01, ***P < .001, or ****P < .0001); non-significant comparisons are not shown. The global group difference was assessed by one-way ANOVA

Affected genes

FH-causing variants were identified in only 2 of the 23 genes studied: 230 individuals (81%), carried P/LP variants in *LDLR* and 53 in *APOB* (19%). No P/LP variants were detected in the *PCSK9* gene. Patients carrying P/LP *LDLR* variants presented with a median LDL-C concentration of 4.63 mmol/L (179 mg/dL). This was significantly higher than the 4.16 mmol/L (161 mg/dL) observed in cases with *APOB* variants (difference 95% CI: 0.16–0.7, $P \leq .05$, Wilcoxon rank sum test).

A total of 88 unique FH-causing variants were detected, with 96% (85/88) found in the *LDLR* gene (see Supplementary data online, *Table S3*). None of the individuals were homozygous or compound heterozygous carriers of variants. Among these genetic alterations, 93% (82/88) were previously reported in ClinVar, and 82% (67/82) of them were annotated as P/LP variants. The remaining 24% (21/88) were classified according to ACMG/AMP criteria. In total, 28% (25/88) were predicted to be truncating variants and the majority, 67% (59/88), were missense variants. The two most prevalent variants, *LDLR* p.Asp266Glu and *APOB* p.Arg3527Gln, explained 38% (109/283) and the four most common pathogenic variants accounted for nearly half of all FH cases (138/283) (*Figure 3A*). On the other hand, a total of 61% (54/88) of pathogenic variants were detected in single cases only, explaining 19% of identified FH cases (54/283). To assess regional effects of this Bavarian study, allele frequencies of the five most common FH variants were compared to those reported in gnomAD.

Unexpectedly frequent was the variant *LDLR* p.Asp266Glu, with an allele frequency of 0.12%. Its prevalence was 5-times higher in Bavaria than in Northern Germany (0.024%, data derived from the SHIP study,[36] $n = 8230$, provided by Dr. Alexander Teumer), 30-times higher than in Europe (0.004%, gnomAD European cohort of 622 057 individuals), and 40-times higher than the global prevalence (0.003%, gnomAD). Array genotyping revealed a shared haplotype, confirming a founder variant and, based on the genetic length of ancestral haplotypes shared between individuals carrying this variant, we estimated its age. Assuming a 'correlated' genealogy, the variant arose 53.6 generations (CI: 16.6–192.8) or approximately 1072 years ago. Assuming an 'independent' genealogy, it arose 79.7 generations (CI: 68.7–92.6) or approximately 1594 years ago.

Similarly, the *APOB* variant p.Arg3527Gln, the second most common variant in this study (0.09%), was 1.8 times more common in Bavaria than in the general European population. Likewise, a 15-fold and a 5-fold higher frequency of the *LDLR* variants p.Cys27Trp (0.03%) and p.Gly592Glu (0.03%) were found in Bavaria. All these variants were significantly more frequent in the study region than in the gnomAD European cohort. In contrast, the *APOB* variant p.Gln4494del was 4.6-times less frequent in Bavaria than in the European gnomAD cohort. Additionally, these five FH-causing variants appear to be specific to Europe, particularly Bavaria, and were much less common or even absent in other global ancestries, including American, African, East Asian, Middle Asian and South Asian populations. *Table 2* gives an overview of the regional differences of the five variants mentioned.

Table 2 Allele frequencies (AF, %) for the five most frequent VRONI variants across reference populations and cohorts

Variant	EU	AMR	AFR	EAS	SAS	MID	Total gnomAD	VRONI	SHIP
p.Asp266Glu	0.004%	0.00%	0.00%	0.00%	0.00%	0.00%	0.003%	0.12%	0.024%
p.Gly592Glu	0.006%	0.004%	0.00%	0.00%	0.00%	0.00%	0.005%	0.03%	-
p.Cys27Trp	0.002%	0.00%	0.00%	0.00%	0.00%	0.02%	0.002%	0.03%	-
p.Arg3527Gln	0.049%	0.004%	0.01%	0.005%	0.00%	0.00%	0.039%	0.09%	-
p.Gln4494del	0.046%	0.01%	0.01%	0.000%	0.00%	0.00%	0.038%	0.01%	-

Population-specific AFs were obtained from gnomAD v4.1; 'Total gnomAD' represents the overall AF across all gnomAD samples. AFs observed in the VRONI screening cohort, and where available in the SHIP population cohort, are shown for comparison. Variants are listed in HGVS protein notation.
EU, Non-Finnish European; AMR, Admixed American; AFR, African/African American; EAS, East Asian; SAS, South Asian; MID, Middle Eastern. '–', data not available.

Discussion

Universal FH screening in children is recommended by numerous authorities.[22,37–41] However, most countries do not yet have such programmes in place. Against this background, and to pave the way for a universal FH screening in Germany, we screened almost 25 000 children for monogenic FH in a population-oriented screening study and made the following observations. Firstly, a single blood collection from the fingertip, obtained by paediatricians during a routine visit, is sufficient to measure LDL-C concentration biochemically and, if indicated, to perform a genetic testing. Secondly, next generation sequencing detected significantly more cases than a dedicated focused panel approach. The progressive classification of newly identified variants is one possible contributing factor. In fact, 358 additional variants have been annotated as P/LP in ClinVar in the last 3 years alone. Thirdly, comparing our data with those from other large-scale European sequencing projects we observed a profound geographical variation of respective FH-causing variants. Indeed, we detected a founder variant in Bavaria which resulted in a FH prevalence that was higher than expected. Fourthly, when screening is not performed as genuine universal screening, ascertainment bias is likely. The relatively high FH prevalence observed in our sample was partly driven by preferential inclusion of children for whom paediatricians had a clinical suspicion, despite our encouragement to conduct a universal screening. Notably, paediatricians who recruited fewer children tended to include a higher proportion of monogenic FH cases, indicating ascertainment bias. Based on 120 000 annual births in Bavaria (between 2010 and 2020) and a recruitment age range of 4.8–14.9 years VRONI enrolled 2.1% of the target population during the study period.

Measurement of LDL-C concentration alone is not sufficient for discriminating various forms of hypercholesterolaemia (e.g. multifactorial or secondary causes). Scores propose clinical stigmata and family history in conjunction with a markedly elevated LDL-C plasma concentration (> 4.14 mmol/L or >160 mg/dL) for diagnosis of FH in children.[20] We have recently demonstrated that approximately half of the children with genetically confirmed FH are not tested positive with recommended FH scores (false negatives).[8] On the other hand, in the same cohort, no FH-defining variant was found in one third of children with a clinical diagnosis according to the FH scores. The therapeutic implications for the latter group are poorly defined, since most studies on medical treatment of FH in children were based on a genetic diagnosis.[12,42–44] A diagnostic algorithm for FH in children may thus benefit from a combined biochemical and molecular diagnostic approach, as recommended by international expert bodies.[22,38,40,45] Specifically, the integration of genetic testing into the screening strategy represents the most effective means of detecting true-positive FH cases while minimizing both false positives and false negatives.[8,19,20,46]

Genetic diagnosis also informs therapy. Medical treatment is recommended for hypercholesterolaemic children with heterozygous FH, for whom statins are available from the age of 6.[47–49] This study tested a centralized approach of screening children from a single capillary blood sample acquired in primary care. Genetic testing was triggered by LDL-C concentrations of ≥3.36 mmol/L (≥130 mg/dL), with the diagnostic yield increasing from 4.7% at 3.36–3.49 mmol/L (130–135 mg/dL) to 78.6% above 5.17 mmol/L (200 mg/dL). However, cases of FH-causing variants below 130 mg/dL were missed, as sequencing all paediatric samples was not feasible. According to published data by Wald *et al.*, the frequency of FH below the 95th LDL-C percentile is ~0.09%, compared to 4.2% above reflecting a 46-fold lower yield.[3] In VRONI, sequencing 519 samples with an LDL-C of 110–130 mg/dL identified two children with FH-causing variants (0.39%). Overall, the diagnostic yield of genetic testing in VRONI was twice that in the paediatric study by Wald *et al.* from 2016, who used a targeted approach analysing the 48 most frequent variants.[3] Simulating the same targeted approach in the VRONI dataset, only 55% (157 of 283) of FH cases—all confirmed by NGS—would have been identified. While a targeted approach may offer reduced costs, it comes at the expense of missed FH diagnoses. FH is also characterized by significant regional variability. The latter is partly due to founder variants, which may influence the FH prevalence in certain populations.[50–52] Such founder variants emphasize the importance of using sequencing methods rather than targeted genotyping approaches when diagnosing FH.

Our hypothesis that *LDLR* p.Asp266Glu, the most prevalent pathogenic variant in our study, has a common ancestral origin was supported by using the haplotype-based age calculation approach of Gandolfo *et al.*[33] which suggests that this variant likely arose several centuries ago and has since been transmitted through multiple generations within this regional population.

Another aspect of FH is the incomplete penetrance of pathogenic variants and the presence of modifying genetic or

environmental factors. Truncating variants are associated with particularly high LDL-C concentrations, whereas missense variants typically have a more modest effect. Notably, LDL-C variability is not confined to differences between variant classes as substantial heterogeneity is evident among individuals with the same variant. For instance, carriers of the *LDLR* variant p.Asp266Glu in the VRONI cohort demonstrated significant variability in LDL-C concentrations (LDL-C range 3.36–6.54 mmol/L or 130–253 mg/dL; *Figure 3B*), confirming that additional genetic and environmental modifiers play a substantial role in determining the phenotype. Overall, pathogenic *LDLR* variants are more prevalent in FH than *APOB* variants, and the higher median LDL-C observed in *LDLR* variant carriers highlights the important role of *LDLR* dysfunction in causing the FH phenotype in this group.

Even though the VRONI screening programme aimed to include all children attending a routine paediatric check-up, recruitment was influenced by clinical decision-making of the primary care paediatricians and/or a pre-selected parental consent. The explicit focus on FH may have raised the awareness of families with a history of hypercholesterolaemia and coronary heart disease (see Supplementary data online, *Figure S2 B, C*). To mitigate the ascertainment bias, we employed a one-stage IPD meta-analysis using a GLMM. After adjusting for practice recruitment rate, the predicted prevalence at screening saturation (rate = 10) was 1 in 163 (0.61%), which compares well with the numbers reported in much larger cohorts such as gnomAD and UK Biobank (*Figure 2*). Further adjusting for the *LDLR* founder variant yielded a background prevalence of 1 in 207, which is consistent with general population estimates in the literature. In conjunction, these data from NGS projects suggest that the FH prevalence is higher than the current consensus of 1 in 250 people, as reported for populations of Western European ancestry.[11,35]

Within Europe, there are only a few countries that carry out genuine, nationwide universal paediatric screening for FH. Slovenia, where it has been implemented since 1995 for approximately 20 000 5-year-old children each year (participation rate of 91%), is a pioneer in this field.[53,54] Nevertheless, various methodological differences limit direct comparability with our findings. Firstly, the first-tier parameter used in Slovenia is total cholesterol rather than LDL-C, as in our approach. Secondly, the LDL-C threshold for the recently implemented extension by NGS genetic testing is higher, which enriched the tested cohort and consequently increased the yield for FH-causing variants among those tested. However, this inevitably reduced case ascertainment overall and resulted in a comparatively low calculated prevalence of FH of 1:409.[55] Even disregarding the Bavarian founder variant, this pattern suggests lower screening sensitivity, indicating that significantly more FH cases may remain undetected than with our approach. While the Netherlands does not carry out nationwide screening of children but has been practising a very effective nationwide cascade screening for decades, other countries (e.g. Czech Republic, Slovakia, Spain, Great Britain) have so far only carried out (partly regional) pilot programmes.

When comparing screening strategies, it is also informative to examine how age influences the diagnostic yield; for example, by comparing adult and paediatric screening strategies. Importantly, the 93rd percentile of LDL-C is substantially different between adults and children (4.91 vs 3.36 mmol/L, or ~190 vs ~130 mg/dL), reflecting the stronger environmental modulation of LDL-C in adults.[56–58] When this 93rd percentile of LDL-C (4.91 mmol/L) was used as the first-tier parameter for selection in sequencing data on adults, as published by Khera *et al*., the diagnostic yield for monogenic FH was approximately 10-fold lower than in the paediatric VRONI cohort (*Figure 1A*).[2] Moreover, 27% of monogenic FH adult cases had LDL-C concentrations <4.91 mmol/L (<190 mg/dL, i.e. < 93rd percentile),[2,58] further indicating that in children LDL-C concentrations may allow for better discrimination of genetically affected individuals. Interestingly, one recent study demonstrated that screening in the age group of 1–12 years offers better discrimination of genetically affected children than screening of newborns, which is consistent with previously published data.[59,60]

Finally, identification of children with monogenic FH is only the first step, which needs to be followed by treatment initiation in the index case as well as reverse cascade screening, identifying an average of two to three additional affected family members per index case. This multiplication effect is critical for maximizing public health impact. Population-based modelling suggests that such strategies could identify up to 70% of all genetically affected individuals within a single generation, underlining the need for a combined screening approach.[61]

In addition to diagnostic yield, acceptability, safety, accessibility and cost are important factors to consider when evaluating a universal FH screening. Integrating screening into routine well-child visits using minimally invasive testing and targeted genetic confirmation promotes high acceptability and safety, enabling broad accessibility and efficient use of resources. Alongside clinical efficacy, the economic feasibility of a universal screening must be considered. While a formal health economic evaluation is beyond the scope of this analysis, the costs and resource requirements associated with identifying FH in this study may provide a context for future universal screening scenarios. Importantly, our first screening step was implemented in routine visits at the paediatrician's office, which have a participation rate of >95% in Germany. A blood test during these visits can be reimbursed by compulsory insurance. Thus, the part of the screening programme which involves all children and indeed was the most expensive part in a recent cost-effectiveness study, could be integrated in current practice with relatively little extra costs.[25] In the VRONI study, the cost of identifying each child with an FH-causing variant was <10 000 €. This includes an enrolment fee for paediatricians, study materials, postage, laboratory costs and, if applicable, genetical analyses cost, as well as study personnel costs. However, cost-effectiveness is highly context-dependent and sensitive to unit costs, compliance, and reimbursement structures. In this context, model calculations within European healthcare systems show benefits to universal screening of young adults, on the other hand calculations in the US demonstrate a wide range of cost-variation, depending on baseline assumptions and costs.[62]

VRONI demonstrates the feasibility of a two-step screening approach for FH identification at the primary care level and provides a framework for future implementation strategies. Key challenges remain, including consistent treatment initiation, long-term adherence, and the systematic expansion of reverse cascade screening, as well as harmonization of genetic counselling pathways and sustainable follow-up structures within national healthcare systems. Within VRONI, next steps focus on

longitudinal outcome evaluation, expansion of cascade screening, and collaboration with national stakeholders to inform future universal FH screening strategies. At the national level, VRONI provides important insights as a scalable pilot model, supporting health policy decisions on integrating paediatric FH screening into routine care as a cornerstone of early cardiovascular prevention.

Limitations

Our study is subject to certain inherent limitations. First, the predetermined LDL-C threshold of 3.36 mmol/L (130 mg/dL) for genetic testing prohibits identification of variant carriers with LDL-C concentrations below this level. This approach was predicated on limited financial resources available in VRONI and the fact that usually an LDL-C of 3.49 mmol/L (135 mg/dL) is the threshold for starting lipid-lowering therapy in children with FH. Second, the voluntary participation of patients allowed local paediatricians to exercise flexibility in the enrolment of children. Some paediatricians included only small numbers of children, which was likely influenced by their clinical judgement (e.g. family history or other clinical criteria), and thus presumably resulted in a selection bias with a condensation of FH cases, i.e. an apparently higher FH frequency in VRONI than in the overall population. On the other hand, the experience made in our voluntary programme—placed in a real-world setting of patient care—comes with the advantage to provide estimates on the numbers of cases that can be effectively identified by a universal FH screening. Third, further studies need to address how the implications of a genetic diagnosis of FH in children are being implemented with respect to guideline-recommended therapy and cascade screening in the affected families. Finally, we did not analyse whether concerns regarding potential harms of a universal screening, such as stigma or parental anxiety, may have affected families or physicians, and how these were weighed against the health benefits coming with the diagnosis of a treatable disease. Recent studies have shown that over 95% of parents support genetic testing when it is offered. Any potential downsides need to be mitigated through structured counselling, clear communication and integration into existing paediatric care structures.[19]

Conclusions

The findings from the VRONI study suggest that universal genetic screening for FH in childhood is technically feasible at the primary care level and diagnostically efficient using a single blood collection. The combined measurement of LDL-C concentration and NGS enabled the identification of FH-affected children at a substantially higher frequency compared to prior screening programmes relying solely on common variant panels or clinical scoring systems. Studying children with LDL-C concentrations above the threshold of 3.36 mmol/L (130 mg/dL), 17% received a definitive genetic diagnosis—approximately three times the yield reported by earlier population-based initiatives in children and ten times higher than in adult cohorts. The calculated prevalence below 1:200 aligns with international NGS-based estimates and may require a correction of currently accepted numbers. Together, these results lend further support to implementation of a structured, sequencing-based FH screening strategy in children as part of national preventive health programmes—both from a medical and public health perspective.

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Authors' contributions

This manuscript was the result of a collaborative effort involving LDS, RSS, HP and VS who received critical input from RS and HS. LDS coordinated and interpreted the genetic analyses. VS and RSS coordinated the acquisition of data and samples. Data visualization and statistical analyses were performed by LDS, RSS and AA. All authors were significantly involved in the VRONI study, which was originally designed by HS and VS.

Supplementary data

Supplementary data are available at *European Heart Journal* online.

Declarations

Disclosure of Interest

MS reports grants from HORIZON/EU, Corona Foundation, BMBF/DZHK, DZG Innovation Fund, and Fondation Leducq. RS reports lecture honoraria and travel support from Chiesi. SH reports institutional grants, consulting fees, lecture honoraria, and advisory board participation with diagnostics and pharmaceutical companies. SA reports lecture honoraria from Infectopharm, Daiichi-Sankyo, Danone-Nutricia, and Novartis. OK reports lecture honoraria from Sanofi and Daiichi Sankyo. WK, TM and HS reports institutional grants as well as personal consulting and lecture fees from multiple pharmaceutical companies. VS reports lecture honoraria from Sanofi, Amgen, Novartis, Lilly and Daiichi Sankyo. All other authors declare no conflicts of interest.

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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Ethical Approval

The study was approved by the ethics committee of the Technical University of Munich.

Pre-registered Clinical Trial Number

German Clinical Trials Register: DRKS00022140.

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