




Population-based screening in children for early diagnosis and treatment of familial hypercholesterolemia: design of the VRONI study

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Background: Heterozygous familial hypercholesterolemia (FH) represents the most frequent monogenic disorder with an estimated prevalence of 1:250 in the general population. Diagnosis during childhood enables early initiation of preventive measures, reducing the risk of severe consecutive atherosclerotic manifestations. Nevertheless, population-based screening programs for FH are scarce. **Methods:** In the VRONI study, children aged 5–14 years in Bavaria are invited to participate in an FH screening program during regular pediatric visits. The screening is based on low-density lipoprotein cholesterol measurements from capillary blood. If exceeding 130 mg/dl (3.34 mmol/l), i.e. the expected 95th percentile in this age group, subsequent molecular genetic analysis for FH is performed. Children with FH pathogenic variants enter a registry and are treated by specialized pediatricians. Furthermore, qualified training centers offer FH-focused training courses to affected families. For first-degree relatives, reverse cascade screening is recommended to identify and treat affected family members. **Results:** Implementation of VRONI required intensive prearrangements for addressing ethical, educational, data safety, legal and organizational aspects, which will be outlined in this article. Recruitment started in early 2021, within the first months, more than 380 pediatricians screened over 5200 children. Approximately 50 000 children are expected to be enrolled in the VRONI study until 2024. **Conclusions:** VRONI aims to test the feasibility of a population-based screening for FH in children in Bavaria, intending to set the stage for a nationwide FH screening infrastructure. Furthermore, we aim to validate genetic variants of unclear significance, detect novel causative mutations and contribute to polygenic risk indices (DRKS00022140; August 2020).

Introduction

Familial hypercholesterolemia (FH) is an autosomal-dominant, inherited disorder of the lipid metabolism, characterized by a substantial elevation of low-density lipoprotein cholesterol (LDL-C) plasma levels due to reduced clearance from the blood. The elevation of LDL-C is already evident in early childhood, causing premature atherosclerotic plaque development and coronary artery disease in the majority of affected individuals.^{1,2} Homozygous FH is quite uncommon (1:500 000) in contrast to

heterozygous FH, which is listed as the most frequent monogenic disorder. Data from Denmark, Netherlands and Norway show an estimated prevalence of 1:250 in the general population, suggesting a similar situation in Germany.^{3,4} Monogenic FH is mainly caused by mutations in the LDL receptor (*LDLR*) gene, resulting in a markedly decreased rate of LDL-C clearance. To a lesser extent, FH results from mutations in a number of other genes, including the apolipoprotein B (*APOB*) gene, reducing the binding affinity of LDL-C to its receptor *LDLR*, and the proprotein convertase subtilisin/kexin type 9 (*PCSK9*) gene, increasing the

degradation of LDLR in hepatocytes in case of gain-of-function mutations.⁵

According to European guidelines, statin therapy is recommended for FH patients, starting at the age of 10 years, along with dietary and lifestyle advice.⁶ Nevertheless, FH remains severely underdiagnosed and undertreated worldwide. Childhood is the optimal period for FH screening, because due to minimal dietary and hormonal influences, LDL-C levels in children reflect predominantly the genetic component and are well suited to discriminate FH from other causes of elevated LDL-C.⁷

If FH remains untreated in this latent stage of the disease, individuals show a 10-fold increase of cardiovascular risk during early and middle adulthood.^{7–9}

Therefore, the most effective approach for detecting FH seems to be a population-based screening during childhood or in young adolescents in combination with reverse cascade screening of first-degree relatives of FH patients.¹⁰ However, such screening raises a number of ethical, legal and logistic issues. Moreover, data safety aspects may raise concerns in asymptomatic children. Here, we describe a screening program for FH in children at the age of 5–14 years. Particularly, we discuss our efforts to overcome ethical, legal, organizational as well as data safety issues. Moreover, our initial experiences and results in the early recruitment phase will be reported.

Methods

The VRONI study is part of DigiMed Bayern, a pilot project in predictive, preventive, personalized and participatory (P4) medicine in Germany, funded by the Bavarian State Ministry of Health and Care. For establishing the VRONI screening program, several fundamental factors were considered, including logistics and infrastructure, ethical, political and legal requirements, general acceptance and practicability at the doctor's office, data security and privacy, sustainability, and cost efficiency.^{11–15} VRONI was designed as a proof-of-concept study, providing a population-based screening program for FH in children in Bavaria. The conceptual overview of the VRONI study is provided in [figure 1](#), and further information can be found on www.myVRONI.de.

Screening and recruitment

Germany offers a pediatric screening program encompassing structured preventive medical examinations during childhood and youth, several with mandatory character, which are conducted in pediatric practices. The program regularly checks the cognitive and bodily development of the child and offers an early detection of uncommon metabolic disorders and other diseases.¹⁶ However, LDL-C measurements are not part of this routine. To increase the efficiency, acceptance and practicability, VRONI is piggybacking on these routine pediatric examinations.

Upon contract with VRONI, the Bavarian Professional Association of Pediatricians (BVKJ) invited all pediatric doctors to enroll individuals. Necessary laboratory materials and documents were provided from the VRONI main office at the Deutsches Herzzentrum München (DHM). Participating pediatricians offer voluntary enrollment in the context of German preventive examinations or as part of any visit of patients aged 5–14 years ([Supplementary figure S1](#)). The child and the parents or legal guardians receive VRONI-specific information material and the attending pediatrician also explains the study and answers arising questions. After written informed consent is obtained, a blood sample of the child as well as relevant baseline data and the family history are collected. Filled-in questionnaires and blood

samples are sent by mail to the VRONI main office for further evaluation.

Data protection and IT infrastructure

Due to the sensitive nature of medical and genetic data, the primary objective is data security and privacy, particularly regarding the identification of personal data. Participant identifying data is only documented on signed consent forms and stored separately in a secured cabinet. All other documents and samples only use a unique, randomly generated, five-character alphanumeric pseudonym. Prefabricated pseudonym barcode labels are used to enable the identification of both medical data and blood samples.

The VRONI database was carefully developed in an iterative process based on a modular design and allows collection, processing, and integration of large amounts of disparate data. To maximize process security and efficiency as well as output, it includes the use of various IT technologies including statistical and analytical tools, including machine learning (ML) and knowledge management systems.

Research data are collected according to the specifications of the Technology and Methods Platform for networked medical research registered association (TMF) guidelines 2017 for data protection in medical research projects including two-stage pseudonymization. All analytical process steps are thus performed exclusively on pseudonymized data. The VRONI database and infrastructure are hosted in a secure container environment on university servers of the DHM in strict adherence to all data safety protocols and regulatory requirements.

Screening for individuals at risk

EDTA blood samples (200- μ l capillary or 1.2-ml venous blood) are sent to the VRONI main office at the DHM by mail. LDL-C measurement is done at the Institute of Laboratory Medicine of the DHM with a quantitative homogeneous enzyme colorimetric method. After centrifugation (2800 \times g, 15 min, 20°C), LDL-C measurement of K3 EDTA plasma (80 μ l) is performed by using the LDL-C test (Roche Diagnostics GmbH, Mannheim, Germany) with the cobas c 501 instrument (Roche Diagnostics GmbH, Mannheim, Germany). The quality of the measured LDL-C result is assessed by determination of the icterus, hemolysis and lipemia indices, using the Serum Index Gene 2 test (Roche Diagnostics GmbH, Mannheim, Germany). Second samples are requested for cases with invalid serum indices.

In case of insufficient sample volume, 40 μ l K3 EDTA plasma is diluted 1:2 with 0.9% sodium chloride solution (B. Braun Melsungen AG, Melsungen, Germany) before measuring the LDL-C concentration. The suitability of this method was validated previously (data not shown); however, determination of serum indices is not possible in diluted samples. Second samples are requested for cases with plasma volumes under 40 μ l.

After established quality control procedures, LDL-C results are transmitted to the VRONI main office. The residual blood clot is resuspended in 100 μ l 1 \times phosphate-buffered saline (Carl Roth GmbH + Co. KG, Karlsruhe, Germany), transferred to a 300 μ l 2D code FluidX Cryo Tube (Brooks, Manchester, UK) and stored at -80° C until potential genetic analysis.

Genetic analysis

In case of clinical suspicion, guidelines recommend to confirm FH genetically by the detection of causative variants.⁶ In addition to providing an unambiguous diagnosis of FH, genetic confirmation can further improve patient management and helps identifying at-risk first-degree relatives via cascade screening.

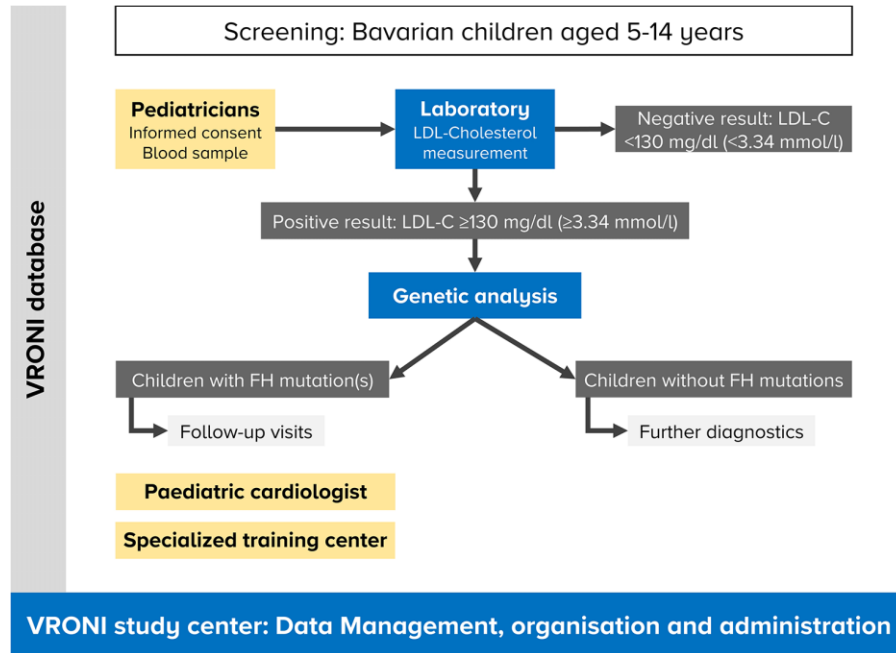


Figure 1 VRONI study—schematic overview. Flowchart of the study: all children aged 5–14 years living in Bavaria are offered a FH screening in the context of routine visits at their pediatrician. A blood sample will be taken and LDL-C centrally measured. In case of LDL-C levels above the 95th percentile, molecular genetic analysis for FH will be performed. Positive tested children and their parents will be informed comprehensively about FH and referred to a specialized training center. Children with elevated LDL-C levels but without known pathogenic FH mutations will undergo further diagnostics, including screening for secondary causes of hypercholesterolemia (e.g. obesity).

In case of an LDL-C ≥ 130 mg/dl (3.34 mmol/l), a conservative threshold in the range of the 95th percentile based on German and Dutch national survey data,^{17,18} the blood clots are sent to the Institute of Neurogenomics (ING) at the Helmholtz Zentrum München for molecular analysis, which is performed on a NovaSeq 6000 (Illumina, CA, USA). A targeted Next-Generation-Sequencing-panel (TWIST Bioscience, CA, USA), based on DNA from the cellular fraction of the initial capillary blood sample, is utilized for genetic testing. This customized FH-panel contains the exonic regions of 23 genes involved in the lipid metabolism (Supplementary table S1). In particular, the entire genomic region of the *LDLR*, *APOB* and *PCSK9* are sequenced, including promoter and intronic regions, excluding repetitive intronic regions, allowing the detection of potentially disease-relevant non-coding variants. Panel sequencing provides at least a 1000-fold coverage of the target region. Sequencing reads are mapped to human genome build GRCh37/hg19. For the analysis and clinical interpretation of sequencing data, the ING cooperates with the Institute of Human Genetics (IHG) at the Technische Universität München (TUM). In detail, the genomic database *ClinVar*¹⁹ and the Exome Variant Annotation Database (EVAdb) at the TUM are utilized. GnomAD is applied for the frequency examination of variants.²⁰ FH is primarily defined as an elevated LDL-C level (≥ 130 mg/dl) in conjunction with pathogenic variants in the *LDLR*, *APOB*, *PCSK9* and *LDLRAP1* (low-density lipoprotein receptor adapter protein 1) genes. Genetic variants are identified either by classification as ‘likely pathogenic’ or ‘pathogenic’ in *ClinVar* and an allele frequency below 0.1%, or by a loss-of-function mutation (i.e. stop mutation, frame-shift mutation, canonical splice shift mutation or large deletion) according to the American College of Medical Genetics and Genomics 2015 guideline.²¹

About 2–4 months after enrollment, the scientific genetic report containing the findings of known pathogenic variants in the *LDLR*, *APOB*, *PCSK9* and *LDLRAP1* genes is sent to the pediatrician, who will inform the family about the results. On this occasion, a second blood sample (2.7-ml venous blood) is obtained. This allows the replication of initial LDL-C measurements and of genetic findings,

resulting in a significant reduction of accidental, undetected sample swapping. Overall, a systematic Failure Mode and Effects Analysis (FMEA) was introduced for minimizing the risk of sample swapping and false results. In children without known pathogenic variants, a repeated LDL-C measurement is initiated and a screening for secondary causes of hypercholesterolemia is carried out. Cases with repeatedly increased LDL-C levels and no genetic and secondary causes identified will be subjected to further experimental approaches.

Experimental approach to uncover new FH mutations

Although an unambiguous diagnosis of FH is based on the detection of pathogenic variants, a causative genetic alteration can most likely only be detected in less than half of patients with clinically elevated LDL-C levels. While the 2019 European Society of Cardiology (ESC)/European Atherosclerosis Society (EAS) guidelines for dyslipidemias mention a detection rate for a genetic cause in 60–80% of clinical FH cases, a Danish study showed rates as low as 20%.^{6,22} This suggests that polygenic causes, statistically adding up many weak effects, may be involved in FH disease development, in addition to cases explained by novel FH mutations in either known or unknown FH genes. Thus, we concomitantly perform chip-based genotyping on a commonly used screening array (GSA; Illumina, CA USA) to assess the polygenic risk score in all patients with elevated LDL-C levels. In a small fraction of FH patients, variants in the non-coding region, causing aberrant splicing and expression of lipid metabolism-associated genes, have been detected.²³ To validate FH-associated variants in non-coding regions functional assays such as RNA sequencing, luciferase assays will be used. Additionally, the functional effects of detected variants with uncertain significance and functionally uncharacterized variants in known FH disease genes are validated *in vitro* using relevant cell lines (CHO-*IdlA7*, HepG2).^{24–26} Whole-exome sequencing in unsolved cases with repeatedly increased LDL-C levels is applied to identify genes not yet associated with FH.

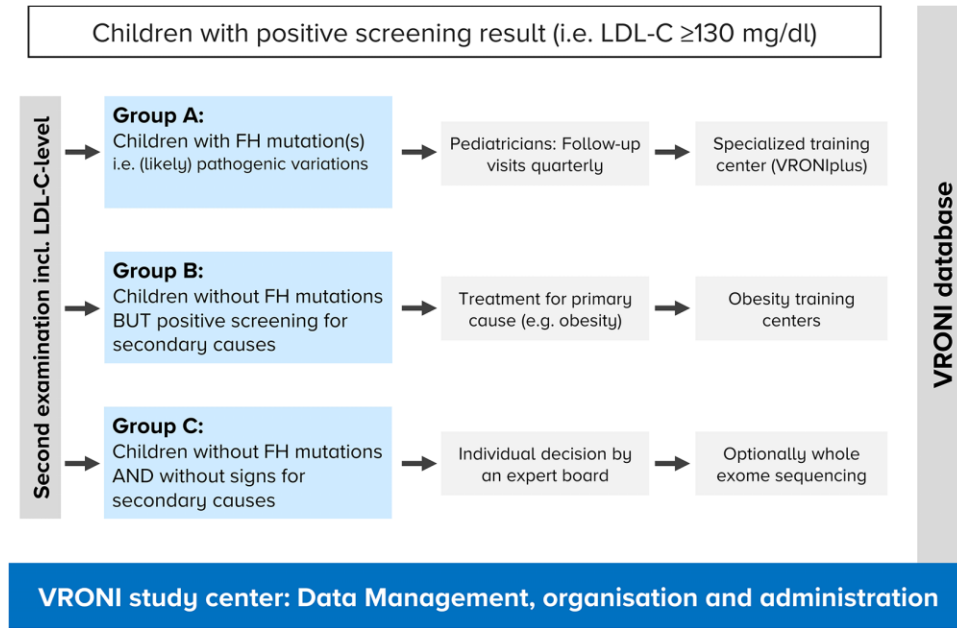


Figure 2 Follow-up in VRONI participants with elevated LDL-C levels. VRONI participants above the LDL-C threshold are subdivided into three distinct groups. Group A (known pathogenic mutations) receive quarterly follow-up visits by specialized pediatricians or pediatric cardiologists and are offered an FH-focused training course at a specialized training center. Group B (no known pathogenic mutation, but evidence for secondary causes of hypercholesterolemia) are recommended to be treated accordingly (e.g. referral to specialized obesity training centers in case of obesity). Group C (no known pathogenic mutation and negative screening for secondary causes) will be reviewed individually by a board of specialists.

Long-term treatment and follow-up

Individuals with confirmed diagnosis of FH will be treated following the guidelines of the ESC and EAS.⁶ VRONI participants with elevated LDL-C levels can be divided into three distinct groups: participants with a known pathogenic genetic variant (Group A); participants without a known pathogenic genetic variant, but with a positive screening for secondary causes of hypercholesterolemia, e.g. obesity (Group B); and participants with neither a known pathogenic genetic variant nor a known secondary cause of hypercholesterolemia (Group C) (figure 2).

For Group A, quarterly follow-up visits (including routine physical and laboratory examinations) are planned, either by the attending pediatrician or the pediatric cardiologist. At baseline, an ultrasound examination of the carotid arteries (including intima media thickness) and echocardiogram are recommended. Results of all follow-up visits are documented in dedicated questionnaires and are sent to the VRONI main office at the DHM. Moreover, affected families are offered an FH-focused training course at a specialized training center (VRONIplus study), educating on FH in a manner suitable for children as well as providing the family with optimized tools to implement and maintain necessary lifestyle changes and pharmacotherapy (Supplementary material).

A reverse cascade screening is strongly recommended and offered to all families in Group A, aiming to detect all affected family members (siblings/children via VRONI and parents/adults in cooperation with CaRe High, an FH Register study in Germany).²⁷

All other participants with elevated LDL-C levels and no pathogenic variant associated (Groups B and C) undergo a second examination in the context of notifying the family about the negative genetic test result. The goal is to check for secondary causes of hypercholesterolemia and to collect data on phenotypical FH criteria. By means of a second blood sample, LDL-C levels are validated approximately 2–4 months after the initial measurement, as generally recommended.^{4,28} Moreover, additional laboratory parameters (to exclude secondary causes) are measured and a dedicated questionnaire about secondary causes and in-depth family history is obtained.

For Group B, no additional follow-ups are scheduled in the context of this study. After identification of the secondary cause for elevated LDL-C levels (obesity, hypothyroidism, nephrotic syndrome, anorexia nervosa, etc.), treatment of the primary cause is organized by the attending pediatrician. In case of obesity, we recommend referring the children to specialized obesity training centers (12 centers in Bavaria) and specific counseling centers to implement and maintain the required lifestyle and nutritional modifications (figure 3).

In Group C, each case will be reviewed individually by a board of experts—encompassing specialists in the fields of cardiology, lipidology, genetics and pediatric cardiology. Recommendations concerning follow-up visits, further diagnostics and therapy will be determined by the board of experts on a case-to-case basis. In selected cases, entire exome sequencing or functional analysis will be performed. In either case, a lifestyle modification is medically indicated.

Results

Within the first months, more than 380 pediatricians subscribed to the VRONI study, almost representing a third of all pediatricians in outpatient offices in Bavaria. Starting in early 2021, around 5200 children were screened for FH by end of August 2021. The anthropometric data as well as the average LDL-C levels are listed in Supplementary table S2, subdivided into age groups. In 48.9% of the participants were female and 51.1% male, the mean age was 9.4 (\pm 3.1) years, and the mean LDL-C was 93.0 (\pm 26.9) mg/dl. Figure 4 shows the distribution of LDL-C levels on all included subjects, of which 8.0% exceeded the predefined LDL-C threshold of 130 mg/dl (3.34 mmol/l). Overall, these numbers are in line with our expectations regarding the prevalence of elevated LDL-C levels in Bavaria.

Discussion

Starting in early 2021 the VRONI study is the first population-based screening program for the early detection of FH associated with

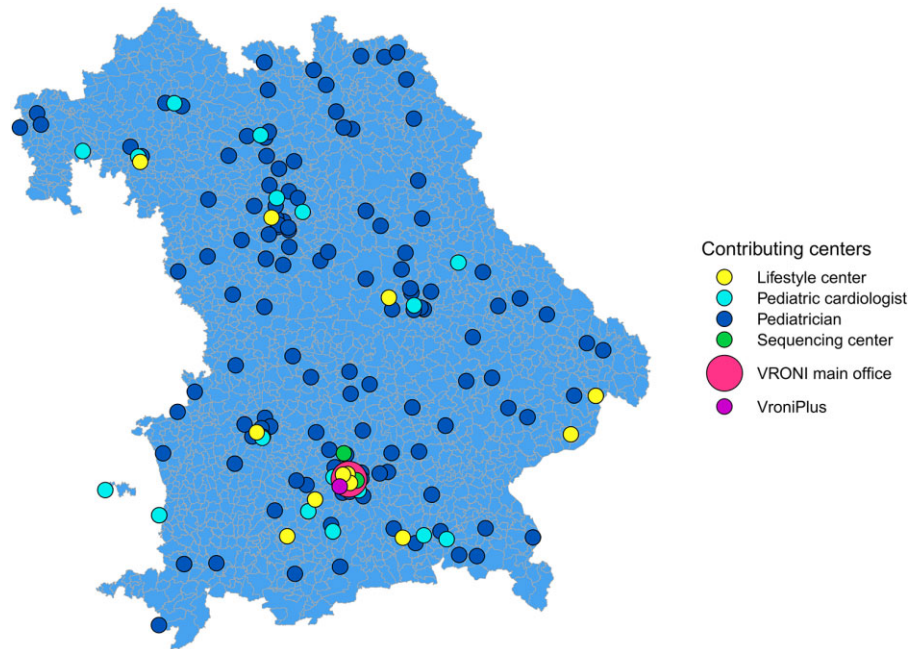


Figure 3 Overview of the VRONI infrastructure in Bavaria. Distribution of contributing centers of the VRONI study in Bavaria. VRONI main office in pink, sequencing center in green, participating pediatricians in dark blue and pediatric cardiologists in light blue, preventive lifestyle centers in yellow and the VroniPlus training center in purple.

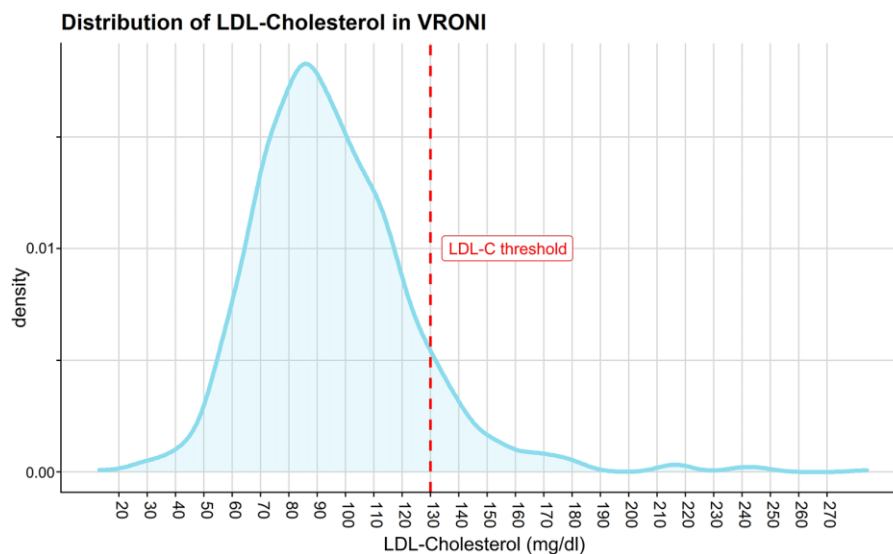


Figure 4 Distribution of LDL-cholesterol measurements in VRONI. Distribution of LDL-cholesterol levels of the first 5200 VRONI participants in Bavaria, Germany. The vertical line represents the predefined LDL-C threshold of 130 mg/dl (3.34 mmol/l).

genetic mutations in children in Southern Germany. The subscription of currently more than 380 pediatricians suggests a high general interest in the topic. Likewise, the rapidly increasing number of participating children indicates the feasibility of the approach. It is aimed to enroll 50 000 children and younger adolescents within a period of 3 years.

The development and design of the VRONI study is a crucial component in the comprehensive model of care for FH, which aims to provide a standardized, high-qualitative and health-economically cost-efficient system of care that is likely to reduce cardiovascular diseases and have the highest impact on patient outcomes. The findings can be used to inform healthcare regulators, insurance policies and coverage decisions for FH genetic testing, recommendations for clinical practice and guidelines to improve the management of FH. Furthermore, in concordance with the primary aims, we will specifically address a number of research

outcomes comprising genetic predispositions, clinical features, health economics management, treatment and outcomes (Supplementary table S3).

Recent results of an International Pediatric FH Register [funded by the International Atherosclerosis Society (IAS)], including country-specific as well as common characteristics of the management of FH in childhood across Europe, show that the majority of children are lacking the full benefit of early FH identification, namely guideline-conform therapy early on, combined with achievement of appropriate lipid-lowering.²⁹ Individuals with FH in particular benefit from early and successful lowering of LDL-C levels, which significantly reduces the risk of serious long-term health effects up to premature death. Hence, especially pediatricians need to be familiar with the diagnosis, treatment and management of FH.³⁰ Moreover, only a small percentage of FH cases are diagnosed, underlining the necessity for a systematic screening. Unfortunately, the

UK National Screening Committee rejected universal child-parent screening for FH in April 2020 in the UK due to 'lack of evidence' [i.e. insufficient evidence about screening-benefit in the prevention of heart diseases due to (I) unclear preventive advantage, (II) unclear suitable age for screening, (III) ethical concern for children of age 2 years and below and (IV) more research required for older children] to recommend population-based screening for FH, although according to contradictory views the approach would yearly prevent 4000 myocardial infarctions, about half of which are fatal, in patients under 50 years of age within the UK.³¹ In contrast, Slovenia leads the way with an established, universal, routine FH screening program in three steps since 2017: (i) universal total cholesterol measurements in 5-year-olds, (ii) followed by genetic testing in case of relevant hypercholesterolemia and (iii) reverse cascade screening of parents in case of FH.¹⁵

Internationally, FH is almost exclusively detected and diagnosed via clinical diagnostic criteria, which are devised on the basis of lipid clinic registries (Simon-Broome diagnostic criteria,³² Dutch Lipid Clinic Network criteria,³³ Make early Diagnosis to Prevent early Deaths³⁴ or Japanese Atherosclerosis Society³⁵). However, these scores are not valid in children as clinical features are quite uncommon at that age and first-degree relatives are comparatively young and thus atherosclerotic manifestation scarce.⁷ In consequence FH screening in children requires a different strategy. In this context, VRONI also aims to identify and assess additional clinical characteristics to facilitate the diagnosis of FH and support the establishment of a children-specific clinical score.

Combining recent advancements in the field of digital technology (e.g. ML) based on big data (electronic health records, FH registries, clinical databases), it seems feasible to establish efficient tools for FH detection and implementation in public health systems with relatively low efforts. Validation and implementation of practicable systematic screening approaches is an important challenge that needs to be addressed. Akyea et al.³⁶ recently published results on a diagnostic approach using ML algorithms, offering a new method (in addition to standard prediction modeling) with a high accuracy in detecting FH in adults. However, these results cannot be directly applied to children, which would be the optimal age group for FH screening. VRONI collects baseline and long-term follow-up data of children with FH and their affected relatives, covering clinical data in form of dedicated questionnaires and health records, laboratory parameters as well as genetic data. Along the same lines, VRONI shall provide multivariate 'big data' on FH, offering the opportunity to develop new approaches for FH screening and management, for example a children-focused ML algorithm for detecting FH. Furthermore, the established infrastructure of VRONI facilitates the assessment of future ML algorithms or other diagnostic tools in a real-world setting, providing feedback on the feasibility and acceptability of these tools.

VRONI offers genetic testing and counseling of families affected by FH, which is strongly recommended as a fundamental part of FH diagnosis in recent guidelines.³⁷ Cascade screening is an effective and proven way to detect affected relatives, thus increasing the overall percentage of FH patients receiving lipid-lowering therapy and consequently reducing morbidity and mortality of premature cardiovascular diseases.³⁸ Recently published data underline the importance of aggressive cholesterol treatment early on in FH patients, favorably beginning with a high-intensity statin. Only rarely side effects of long-term statin therapy in children and adolescents are reported.^{9,39}

VRONI is embedded in the EAS and Familial Hypercholesterolemia Studies Collaboration (FHSC) which aims to establish a worldwide, large-scale and standardized registry of patients with FH, containing data on detection strategies and the clinical implications thereof.⁴⁰ Alongside the benefits, VRONI also introduces several ethical challenges such as the ethics of participation: Because genetic test results also provide information about close relatives, there are implications for them as well, which

complicate the individual basic right '(not) to know'. We address these issues with an additional sociological research project and investigate how participants and pediatricians actually understand and handle VRONI's chances and challenges.

Summary and conclusions

VRONI is a novel, multidisciplinary model of FH care that is characterized by a unique collaboration between scientific research and clinical practice. Collaborating with primary care pediatricians, VRONI will provide evidence for the feasibility of routine population-based screening for FH in children in Germany. A primary goal is to use molecular and clinical diagnostics to establish systematic efforts to identify and treat patients with FH. Furthermore, the identification of children and parents in the context of cascade screening before the onset of cardiovascular events provides a possibility to initiate preventive medication early and most effectively.

VRONI will collect and analyze an exceptional population-based dataset on individuals with FH and thus will allow realistic estimates on the prevalence of FH in Southern Germany. In addition, VRONI will assist the medical community in care and support of FH patients to supplement clinical guidelines and to develop FH screening programs nationally and internationally. Ultimately, VRONI will help to pose and clear relevant ethical questions in the context of FH.

Ethics approval

The VRONI study protocol was approved by the independent ethical committee of the Technische Universität München and will be conducted in accordance with the provisions of the Declaration of Helsinki and the International Conference on Harmonization Good Clinical Practice guidelines.

Data availability

Individual-level data are not publicly available due to their 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.

Code availability (software application or custom code)

All bioinformatics applications can be requested at the VRONI main office in Munich from the corresponding authors.

Supplementary data

Supplementary data are available at *EURPUB* online.

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Conflicts of interest: None declared.

Key points

- Individuals with familial hypercholesterolemia (FH) benefit from early diagnosis and subsequent treatment, which significantly prevent cardiovascular diseases up to premature death.
- The management of FH is a major public health priority in need of broad recognition and practical implementation in outpatient care.
- VRONI is a novel, multidisciplinary model of FH care that is characterized by a unique collaboration between scientific research and clinical practice.
- VRONI aims to provide a standardized, high-qualitative and health-economically cost-efficient system of care that is likely to reduce cardiovascular diseases.

References

- Goldstein JL, Brown MS. The LDL receptor. *Arterioscler Thromb Vasc Biol* 2009;29:431–8.
- Austin MA, Hutter CM, Zimmern RL, Humphries SE. Genetic causes of monogenic heterozygous familial hypercholesterolemia: a HuGE prevalence review. *Am J Epidemiol* 2004;160:407–20.
- Klose G, Laufs U, Marz W, Windler E. Familial hypercholesterolemia: developments in diagnosis and treatment. *Dtsch Arztebl Int* 2014;111:523–9.
- Nordestgaard BG, Chapman MJ, Humphries SE, et al.; for the European Atherosclerosis Society Consensus Panel. Familial hypercholesterolaemia is underdiagnosed and undertreated in the general population: guidance for clinicians to prevent coronary heart disease: consensus statement of the European Atherosclerosis Society. *Eur Heart J* 2013;34:3478–90a.
- Rader DJ, Cohen J, Hobbs HH. Monogenic hypercholesterolemia: new insights in pathogenesis and treatment. *J Clin Invest* 2003;111:1795–803.
- Mach F, Baigent C, Catapano AL, et al.; ESC Scientific Document Group. 2019 ESC/EAS Guidelines for the management of dyslipidaemias: lipid modification to reduce cardiovascular risk. *Eur Heart J* 2020;41:111–88.
- Wiegman A, Gidding SS, Watts GF, et al. Familial hypercholesterolaemia in children and adolescents: gaining decades of life by optimizing detection and treatment. *Eur Heart J* 2015;36:2425–37.
- Daniels SR, Gidding SS, de Ferranti SD; National Lipid Association Expert Panel on Familial Hypercholesterolemia. Pediatric aspects of familial hypercholesterolemias: recommendations from the National Lipid Association Expert Panel on Familial Hypercholesterolemia. *J Clin Lipidol* 2011;5:S30–7.
- Luirink IK, Wiegman A, Kusters DM, Hof MH, et al. 20-Year follow-up of statins in children with familial hypercholesterolemia. *N Engl J Med* 2019;381:1547–56.
- Wald DS, Bestwick JP, Morris JK, et al. Child-parent familial hypercholesterolemia screening in primary care. *N Engl J Med* 2016;375:1628–37.
- Kordonouri O, Lange K, Boettcher I, et al. New approach for detection of LDL-hypercholesterolemia in the pediatric population: the Fr1dolin-Trial in Lower Saxony, Germany. *Atherosclerosis* 2019;280:85–91.
- Ramaswami U, Cooper J, Humphries SE; FH Paediatric Register Steering Group. The UK paediatric familial hypercholesterolaemia register: preliminary data. *Arch Dis Child* 2017;102:255–60.
- Galema-Boers JM, Versmissen J, Roeters van Lennep HW, et al. Cascade screening of familial hypercholesterolemia must go on. *Atherosclerosis* 2015;242:415–7.
- Bogsrud MP, Langset G, Wium C, et al. Treatment goal attainment in children with familial hypercholesterolemia: a cohort study of 302 children in Norway. *J Clin Lipidol* 2018;12:375–82.
- Grosej U, Kovac J, Sustar U, et al. Universal screening for familial hypercholesterolemia in children: the Slovenian model and literature review. *Atherosclerosis* 2018;277:383–91.
- Gesundheit Bf. Gesundheitsuntersuchungen für Kinder und Jugendliche 2021. Available at: <https://www.bundesgesundheitsministerium.de/themen/praevention/kindergesundheit/frueherkennungsuntersuchung-bei-kindern.html> (accessed 9 October 2021).
- Dortsch R, Rosario AS, Scheidt-Nave C, et al. *Bevölkerungsbezogene Verteilungswerte ausgewählter Laborparameter aus der Studie zur Gesundheit von Kindern und Jugendlichen in Deutschland (KiGGS)*. Robert Koch-Institut, Berlin 2009: 135.
- Balder JW, Lansberg PJ, Hof MH, et al. Pediatric lipid reference values in the general population: the Dutch lifelines cohort study. *J Clin Lipidol* 2018;12:1208–16.
- Landrum MJ, Lee JM, Benson M, et al. ClinVar: improving access to variant interpretations and supporting evidence. *Nucleic Acids Res* 2018;46:D1062–7.
- Karczewski KJ, Francioli LC, Tiao G, et al. The mutational constraint spectrum quantified from variation in 141,456 humans. *Nature* 2020;581:434–43.
- Richards S, Aziz N, Bale S, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genet Med* 2015;17:405–24.
- Benn M, Watts GF, Tybjaerg-Hansen A, Nordestgaard BG. Familial hypercholesterolemia in the Danish general population: prevalence, coronary artery disease, and cholesterol-lowering medication. *J Clin Endocrinol Metab* 2012;97:3956–64.
- Reeskamp LF, Hartgers ML, Peter J, et al. A deep intronic variant in LDLR in familial hypercholesterolemia. *Circ Genom Precis Med* 2018;11:e002385.
- Benito-Vicente A, Uribe KB, Jebari S, et al. Validation of LDLr activity as a tool to improve genetic diagnosis of familial hypercholesterolemia: a retrospective on functional characterization of LDLr variants. *Int J Mol Sci* 2018;19:61676. <https://doi.org/10.3390/ijms19061676>.
- Di Taranto MD, Benito-Vicente A, Giacobbe C, et al. Identification and *in vitro* characterization of two new PCSK9 gain of function variants found in patients with familial hypercholesterolemia. *Sci Rep* 2017;7:15282.
- Fernández-Higuero JA, Etxebarria A, Benito-Vicente A, et al. Structural analysis of APOB variants, p.(Arg3527Gln), p.(Arg1164Thr) and p.(Gln4494del), causing familial hypercholesterolaemia provides novel insights into variant pathogenicity. *Sci Rep* 2015;5:18184.
- Schmidt N, Dressel A, Grammer TB, et al. Lipid-modifying therapy and low-density lipoprotein cholesterol goal attainment in patients with familial hypercholesterolemia in Germany: the CaReHigh Registry. *Atherosclerosis* 2018;277:314–22.
- Watts GF, Gidding S, Wierzbicki AS, et al. Integrated guidance on the care of familial hypercholesterolaemia from the International FH Foundation. *Int J Cardiol* 2014;171:309–25.
- Ramaswami U, Futema M, Bogsrud MP, et al. Comparison of the characteristics at diagnosis and treatment of children with heterozygous familial hypercholesterolaemia (FH) from eight European countries. *Atherosclerosis* 2020;292:178–87.
- Dombalis S, Nash A. The effect of statins in children and adolescents with familial hypercholesterolemia: a systematic review. *J Pediatr Health Care* 2021;35:292–303.
- Wald DS, Martin AC. Decision to reject screening for familial hypercholesterolaemia is flawed. *Arch Dis Child* 2021;106:525–6.
- Mortality in treated heterozygous familial hypercholesterolaemia: implications for clinical management. Scientific Steering Committee on behalf of the Simon Broome Register Group. *Atherosclerosis* 1999;142:105–12.
- Civeira F; International Panel on Management of Familial Hypercholesterolemia. Guidelines for the diagnosis and management of heterozygous familial hypercholesterolemia. *Atherosclerosis* 2004;173:55–68.
- Williams RR, Hunt SC, Schumacher MC, et al. Diagnosing heterozygous familial hypercholesterolemia using new practical criteria validated by molecular genetics. *Am J Cardiol* 1993;72:171–6.
- Harada-Shiba M, Arai H, Ishigaki Y, et al.; Working Group by Japan Atherosclerosis Society for Making Guidance of Familial Hypercholesterolemia. Guidelines for diagnosis and treatment of familial hypercholesterolemia 2017. *J Atheroscler Thromb* 2018;25:751–70.
- Akyea RK, Qureshi N, Kai J, Weng SF. Performance and clinical utility of supervised machine-learning approaches in detecting familial hypercholesterolaemia in primary care. *NPJ Digit Med* 2020;3:142.
- Musunuru K, Hershberger RE, Day SM, et al.; American Heart Association Council on Genomic and Precision Medicine; Council on Arteriosclerosis, Thrombosis and Vascular Biology; Council on Cardiovascular and Stroke Nursing; and Council on Clinical Cardiology. Genetic testing for inherited cardiovascular diseases: a scientific statement from the American Heart Association. *Circ Genom Precis Med* 2020;13:e000067.
- Lee C, Rivera-Valerio M, Bangash H, et al. New case detection by cascade testing in familial hypercholesterolemia: a systematic review of the literature. *Circ Genom Precis Med* 2019;12:e002723.
- Braamskamp M, Langset G, McCrindle BW, et al. Effect of rosuvastatin on carotid intima-media thickness in children with heterozygous familial hypercholesterolemia: the CHARON Study (Hypercholesterolemia in Children and Adolescents Taking Rosuvastatin Open Label). *Circulation* 2017;136:359–66.
- Vallejo-Vaz AJ, Kondapally Seshasai SR, Cole D, et al. Familial hypercholesterolaemia: a global call to arms. *Atherosclerosis* 2015;243:257–9.