Population-Based Screening in Children For Early Diagnosis and Treatment of Familial Hypercholesterolemia: Design of The VRONI Study

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Abstract

**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 to 14 years in Bavaria are invited to participate in a FH screening program during regular pediatric visits. The screening is based on LDL-C 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 organisational aspects, which will be outlined in this paper. Recruitment started in January of 2021, within two months more than 280 pediatricians screened over 1,150 children. Approximately 60,000 children are expected to be enrolled in the VRONI study until 2024.

**Conclusion:** VRONI aims to test the feasibility of a population-based screening for FH in children in Bavaria, intending to set the stage for a nation-wide FH screening infrastructure. Further we aim to validate genetic variants of unclear significance, detect novel causative mutations, and contribute to polygenic risk indices. (German Clinical Trials Register: DRKS00022140; registered August 21st2020.)

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 (CAD) 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 with an estimated prevalence of 1:250 in the general population.(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.(1, 5) 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.(6)

According to European guidelines, statin therapy is recommended for FH patients, starting at the age of 10 years, along with dietary and lifestyle advice.(7, 8) 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.(9)

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.(9-11)

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.(12-14) 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 to 14 years. Particularly, we discuss our efforts to overcome ethical, legal, organisational as well as data-safety issues. Moreover, our initial experiences and results in the early recruitment phase will be reported.

**Materials And 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 lessons learned from similar trials, logistics and infrastructure, ethical, political and legal requirements, general acceptance and practicability at the doctor’s office, governance, data security and privacy, sustainability, and cost efficiency.(15-19) VRONI was designed as a proof of concept study, providing a population-based screening program for FH in children in Bavaria. The overall conceptual overview of the VRONI study is provided in the Graphic Abstract. Further information can be found on www.myVRONI.de.

**Screening and recruitment**

Germany offers a pediatric screening program comprising structured preventive medical examinations during childhood and youth – several with mandatory character. Such examinations are conducted by pediatricians in their out-patient offices. However, LDL-C measurements are not part of this routine. To increase gain in efficiency, acceptance and practicability, VRONI was included in already established 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 (U9, U10, U11, and J1) or as part of any patient visit between 5 and 14 years of age (Figure 1). 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 identification of personal data. The only document with participant identifying data is the signed consent form, stored separately in a secured cabinet. All other documents and samples only use a unique, randomly generated, 5-character alphanumeric pseudonym. Prefabricated pseudonym barcode labels are used to enable 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 and knowledge management systems.

Research data is 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. Furthermore, the IT architecture and platform allow data flow monitoring at various stages and simplify the upkeep of appropriate quality standards. For external access to data, GDPR-compliant (General Data Protection Regulation in its German version) concepts for the exchange of data and samples have been established. The VRONI database and infrastructure is hosted in a secure container environment on university servers of the DHM in strict adherence with all data safety protocols and regulatory requirements. The data protection and security concept were established in concordance with the responsible data protection authorities and local ethics committees and reviewed positively. Beyond compliance necessity, processes and documents were pro-actively and iteratively checked with the Bavarian State Data Protection Officer for advice to secure optimal procedures.

Screening for individuals at risk

EDTA blood samples (200 µl capillary or 1.2 ml venous blood) are sent to the screening center 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 (2,800 x g, 15 min, 20°C), LDL-C measurement of K3 EDTA plasma (80 µl) is performed by using the LDLC3 test (Roche Diagnostics GmbH, Mannheim, Germany) with the cobas c 501 instrument (Roche Diagnostics GmbH, Mannheim, Germany).

At the same time as the sample is processed, the quality of the measured LDL-C result is assessed by determination of the indices of icterus, hemolysis, and lipemia using the Serum Index Gene 2 test (Roche Diagnostics GmbH, Mannheim, Germany). In case of invalid serum indices, a second sample is required by the VRONI main office at the DHM.
In case of insufficient sample volume 40 µl 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 the method was validated previously (data not shown). In this case, the determination of the serum indices is not possible. For samples providing less than 40 µl K3 EDTA plasma a second sample is requested by the VRONI main office.

LDL-C results undergo established quality control procedures and subsequently transmitted to the VRONI main office. After LDL-C determination the residual blood clot is resuspended in 100 µl 1 x phosphate-buffered saline (Carl Roth GmbH + Co. KG, Karlsruhe, Germany), transferred to a 300 µl 2D code FluidX Cryo Tube (Brooks, Manchester, United Kingdom), and stored at -80°C until potential genetic analysis. Blood clots from samples with an LDL-C $\geq$ 130 mg/dl are sent to the Institute of Neurogenomics, Helmholtz Zentrum München (HMGU) for genetic analysis.

**Genetic analysis**

In case of clinical suspicion, guidelines recommend to confirm FH genetically by the detection of causative variants.(7) Despite providing an unambiguous diagnosis of FH, the genetic confirmation of FH further has the potential to improve patient management and identify at-risk first-degree relatives through cascade screening.(20)

Cases exceeding the LDL-C threshold of $>130$ mg/dl ($>3.34$ mmol/l) undergo genetic investigation at the Helmholtz Zentrum München and the Institute of Human Genetics (IHG) at the Technische Universität München (TUM). Sequencing is performed on a NovaSeq 6000 (Illumina, CA USA) at the HMGU. A targeted NGS-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 (Supplemental Table 1). 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. Sequencing reads are mapped to human genome build GRCh37/hg19. For the analysis and clinical interpretation of sequencing data the genomic database ClinVar (21) and the Exome Variant Annotation Database (EVAdb) at the TUM are utilized. GnomAD is applied for the frequency examination of variants.(22) FH is primarily defined as an elevated LDL-C level ($>130$ mg/dl or $>3.34$ mmol/l) in conjunction with pathogenic variants in the \( LDLR, APOB, PCSK9 \) and \( LDLRAP1 \) 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, frameshift mutation, canonical splice shift mutation or large deletion) according to the American College of Medical Genetics and Genomics 2015 guideline.(23)

Despite the progress made in NGS methodologies, a known causative mutation can only be detected in 60-80% of patients with clinically definite or probable FH.(8) Reasons for this could be variants in novel disease genes, epigenetic mechanisms, secondary cause of disease or most importantly a polygenic
background. An increased polygenic risk for FH results from small additive effects of a number of single nucleotide polymorphisms (SNPs) located along the whole genome.

Thus, chip-based genotyping, based on DNA from the cellular fraction of the initial capillary blood sample, is conducted concomitantly to assess the polygenic risk score in all patients with elevated LDL-C levels. A commonly used screening array (GSA, Illumina, CA USA) is used for this purpose.

A scientific genetic report, containing the findings of known pathogenic variants in the LDLR, APOB, PCSK9 and LDLRAP1 (Low-density lipoprotein receptor adapter protein 1) genes is sent to the responsible pediatrician to inform the family about the results. On this occasion, about two to four months after enrollment, 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. Along with the second blood sample, a routine follow-up in children with known pathogenic variants is performed by the pediatrician. In children without known pathogenic variants in known FH disease genes, a screening for secondary causes of hypercholesterolemia is carried out.

**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 only be detected in 60-80% of patients with clinically elevated LDL-C levels. 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.(24) Furthermore, 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.(25) A combination of total mRNA sequencing and whole exome sequencing in unsolved cases with repeatedly increased LDL-C levels is applied to identify FH-associated variants in non-coding regions or genes not yet associated with FH. Consequently, in selected cases, PAX tubes are requested for RNA isolation and venous blood samples are used for exome sequencing. Should non-coding variants be identified in cases with no available RNA sample, functional assays such as luciferase assays are employed for validation.

The growing application of next-generation sequencing (NGS) technologies in routine diagnostics facilitates the rapid identification of pathogenic variants. Currently, almost 3,000 LDLR variants have been described (ClinVar), affecting LDLR activity to various degrees. However, the pathogenicity of the great majority of LDLR variants has not been functionally studied.(26) To ensure early treatment and favorable prognosis, early and definitive diagnosis of FH is important. Functional effects of detected variants with uncertain significance (VUS) and functionally uncharacterized variants in known FH disease genes are validated in vitro using relevant cell lines (CHO-ldlA7, HepG2).(27-29)

**Long-term treatment and Follow-up**
Individuals with confirmed diagnosis of FH will be treated following the guidelines of the European Society of Cardiology (ESC) and European Atherosclerosis Society (EAS). Additionally, 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 are planned, either by the attending pediatrician or a pediatric cardiologist. In addition to the routine physical and laboratory examinations, regular ultrasound examinations of the carotid arteries (including measurements of the intima media thickness) as well as echocardiograms are recommended at baseline. Results of these follow-up visits are documented in dedicated questionnaires and sent to the VRONI main office at the DHM. Moreover, affected families are offered a FH-focused training course at a specialized training center in context of the VRONIplus study, educating in a manner suitable for children as well as providing the family with optimized tools to implement and maintain necessary lifestyle changes and pharmacotherapy (Supplemental Material).

Within the framework of a research project, the genetic analysis in VRONI only qualifies as a scientific report and does not equate legally to a valid medical diagnosis in Germany. Consequently, affected families and their pediatricians are advised to confirm the highly suspected FH diagnosis by conventional focused genotyping in accordance with the German health insurance regulations. Further, independent genetic analysis comes with the advantage of further minimizing any mix-up or analysis errors. On the other hand, patients need to consider, that in Germany a registered genetic diagnosis can discriminate affected individuals regarding future insurance contracts or pursuit of a civil service career path. Alongside the follow-up, 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, a FH Register study in Germany).

All other participants with elevated LDL-C levels and no pathogenic variant associated (Group B and C) undergo a second examination, at the time of notifying the family of the so far 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, the elevated LDL-C levels are validated approximately two to four months after the initial LDL-C measurement, as generally recommended. 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 (e.g. 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 counselling 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 280 pediatricians subscribed to the VRONI study, already representing over a fifth of all pediatricians in out-patient offices in Bavaria. Starting in early 2021 around 1,150 children were screened for FH by mid-February 2021. The anthropometric data as well as the average LDL-C levels are listed in Table 2, subdivided in age groups. 548 participants were female (47%) and 610 male (53%), the mean age was 9.8 (± 3.2) years and the mean LDL-C was 95.3 (± 28.7) mg/dl. Figure 4 shows the distribution of LDL-C levels on the first 1,158 subjects, of which 9.7% 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 genetic mutations in children in Southern Germany. Subscription of currently more than 280 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 enrol 60,000 children and younger adolescents within a period of three years.

We first established an extensive logistic network, spanning the VRONI main office at the DHM, primary care pediatricians located in Bavaria (recruitment centers), transportation of blood samples from all over Bavaria to the Institute of Laboratory Medicine at the DHM and a specialized molecular genetic testing center of the Institute of Human Genetics of the TUM. Further, we established a GDPR compliant data infrastructure allowing to integrate different types of data centrally. Data are constantly monitored for consistency, accuracy and plausibility by a checklist following the criteria of the Federal Emergency Management Agency (FEMA). In doing so, VRONI represents an excellent opportunity to collect epidemiologic and clinical data to tackle the burden of FH and contribute to the prevention of coronary artery disease.

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 (Table 2).

Early diagnosis and subsequent treatment of FH is a major public health priority in need of broad recognition and practical implementation in outpatient care. 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. 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.(32) 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 4,000 myocardial infarctions, about half of which are fatal, in patients under 50 years of age within the UK.(33) The VRONI study aims to contribute considerably to clarify the picture.

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 (34), Dutch Lipid Clinic Network criteria (35), Make early Diagnosis to Prevent early Deaths (36), or Japanese Atherosclerosis Society (37)). However, these scores are not valid in children since clinical features are quite uncommon at that age and first-degree relatives are comparatively young and thus atherosclerotic manifestation scarce.(9) 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. machine learning) 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. (2020) recently published results on a diagnostic approach using machine-learning (ML) algorithms, offering a new method (in addition to standard prediction modelling) with a high accuracy in detecting FH in adults.(38) 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 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 counselling of families affected by FH, which is strongly recommended as a fundamental part of FH diagnosis in recent guidelines.(39, 40) 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.(41) 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.(11, 42)

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.(43) Alongside the benefits, VRONI also introduces several ethical challenges such as the ethics of participation: Since 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 will provide evidence for the feasibility of routine population-based screening for FH in children in Germany, in collaboration with primary care pediatricians. 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.

**Declarations**
Funding

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Conflicts of interest/Competing interests

None.

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.

Consent to participate

For all participants written informed consent was obtained.

Consent for publication

All authors reviewed the manuscript and agreed on publication.

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.

Code availability (software application or custom code)

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

Authors’ contributions

V. Sanin and R. Schmieder drafted the manuscript. H. Schunkert designed the framework of the study. All authors provided intellectual input, critically reviewed the manuscript and agreed to its publication.

Acknowledgement

We want to thank all participating pediatricians and collaborators of the DigiMed Bayern consortium for their excellent support. Additional thanks are due to all involved personal at the VRONI main office, the Institute of Laboratory Medicine at the DHM, the Helmholtz Zentrum München and the Institute of Human Genetics at the TUM for their continuous and excellent work.
References


33. Wald DS, Martin AC. Decision to reject screening for familial hypercholesterolaemia is flawed. Archives of disease in childhood. 2020.


Tables

Table 1: Anthropometric date of the VRONI study

<table>
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<th>Weight (kg)</th>
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Summary of clinical characteristics (n=1158) as well as of LDL-Cholesterol levels in VRONI up to February 2021. Data is presented as mean ± SD.

Table 2. Predefined main research outcomes of VRONI
Clinical features and health economics management

- Data analyses and reporting by the registry on estimated prevalence; geographical distribution, single genetic variants associated with disease and polygenic score
- Identification of clinical criteria helpful for diagnostic of FH
- Evaluation of collaboration between families and physicians
- Value, cost effectiveness and acceptance of a generalized screening
- Cost effectiveness and treatment strategies including interventional approach in context of the reverse cascade screening

Treatment (lifestyle and LDL-C lowering medication)

- Change in lipid parameters
- Change in carotid intima-media thickness
- Change in measures of growth and maturation
- Identification of parameters to monitor the treatment
- Role of additional lifestyle risk factors (as obesity, lack of movement, high blood pressure and smoking) in prevention
- Analyses of long-term side effects under treatment with statins or ezetimibe
- Long term- cost and benefit analysis of the treatment

Patient outcomes

- Screening and treatment effects in long term risk of cardiovascular events
- Quality of life assessment
- Treatment compliance and adherence

Overview of the predefines main aims of the VRONI study.

Figures
The FH screening by the VRONI Study is integrated in an established children examination protocol. The figure shows the recommended preventive pediatric visits in Germany. The VRONI Screening period (U9-J1) and the age-specific LDL-C levels measured in the first 1158 participants (median, 5th and 95th percentile) are outlined in red.

**Figure 1**

<table>
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<th>Preventive pediatric visits</th>
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<td>postpartum</td>
<td></td>
<td></td>
</tr>
<tr>
<td>U2</td>
<td>3-10 days</td>
<td></td>
<td></td>
</tr>
<tr>
<td>U3</td>
<td>4-5 weeks</td>
<td></td>
<td></td>
</tr>
<tr>
<td>U4</td>
<td>3-4 months</td>
<td></td>
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</tr>
<tr>
<td>U5</td>
<td>6-7 months</td>
<td></td>
<td></td>
</tr>
<tr>
<td>U6</td>
<td>10-12 months</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Toddler/Prechooler</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>U7</td>
<td>21-24 months</td>
<td></td>
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</tr>
<tr>
<td>U7a</td>
<td>34-36 months</td>
<td></td>
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</tr>
<tr>
<td>U8</td>
<td>46-48 months</td>
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</tr>
<tr>
<td>U9</td>
<td>60-64 months</td>
<td></td>
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<tr>
<td>School child</td>
<td></td>
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</tr>
<tr>
<td>U10</td>
<td>7-8 years</td>
<td></td>
<td></td>
</tr>
<tr>
<td>U11</td>
<td>9-10 years</td>
<td></td>
<td></td>
</tr>
<tr>
<td>J1</td>
<td>12-14 years</td>
<td></td>
<td></td>
</tr>
<tr>
<td>J2</td>
<td>16-17 years</td>
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</tbody>
</table>
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 a 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.
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 1158 VRONI participants in Bavaria, Germany. The vertical line represents the predefined LDL-C threshold of 130 mg/dl (3.34 mmol/l).

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Suppl.VRONIpluspatienteducationprogramme.docx
- SupplementalBavarianPediatriciansConsortium.docx
- SupplementalDigiMedBayernConsortiumCoauthorlist.docx
- SupplementalInformedConsentForm1.pdf
- SupplementalTUMEthicsapproval1.pdf
- SupplementalTable1..pdf
- GraphicAbstract..pdf