BMJ Open

Screening for familial hypercholesterolaemia in primary school children: protocol for a cross-sectional, feasibility study in Luxemburg city (EARLIE)

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2022-066067
Article Type:	Protocol
Date Submitted by the Author:	03-Jul-2022
Complete List of Authors:	Becker, Marianne; Centre Hospitalier de Luxembourg, Pediatric endocrinology and diabetology (DECCP); VUB University, Research Group GRON Adamski, Aurélie; Centre Hospitalier de Luxembourg, Pediatric endocrinology and diabetology (DECCP) Fandel, Françoise; City of Luxembourg, Department of School Medicine Vaillant, Michel; Luxembourg Institute of Health, Competence Centre for Methodology and Statistics Wagner, Kerstin; Centre Hospitalier de Luxembourg, Department of paediatric cardiology Droste, Dirk; Centre Hospitalier de Luxembourg, Department of Neurology Ziade, Bechara; Luxembourg Ministry of Health, Direction Hein, Steve; Centre Hospitalier de Luxembourg, Department of sports medicine Mendon, Priyanka; Luxembourg Institute of Health, Competence Center for Methodology and Statistics Bocquet, Valéry; Luxembourg Institute of Health, Competence Center for Methodology and Statistics de Beaufort, Carine; Centre Hospitalier de Luxembourg, Department of paediatric endocrinology and diabetology (DECCP); University of Luxembourg - Belval Campus, Faculty of Science, Technology and Medicine
Keywords:	Lipid disorders < DIABETES & ENDOCRINOLOGY, Paediatric endocrinology < DIABETES & ENDOCRINOLOGY, Community child health < PAEDIATRICS, PUBLIC HEALTH, VASCULAR MEDICINE

Screening for familial hypercholesterolaemia in primary school children: protocol for a cross-sectional, feasibility study in Luxemburg city (EARLIE)

Marianne Becker[1,9], Aurélie Adamski[1], Françoise Fandel[2], Michel Vaillant[3], Kerstin Wagner[4], Dirk W. Droste[5], Bechara Ziade[6], Steve Hein[7], Priyanka Mendon[3], Valéry Bocquet[3], Carine de Beaufort[1,8]

[1] Department of paediatric endocrinology and diabetology (DECCP), Centre hospitalier de Luxembourg, Luxembourg

[2] Department of School Medicine, City of Luxembourg, Luxembourg

[3] Competence Center for Methodology and Statistics, Luxembourg Institute of Health, Luxembourg

[4] Department of paediatric cardiology, Centre hospitalier de Luxembourg, Luxembourg

[5] Department of Neurology, Centre Hospitalier de Luxembourg, Luxembourg

[6] Direction of the ministry of Health, Luxembourg

[7] Department of sports medicine, Centre hospitalier de Luxembourg, Luxembourg

[8] Faculty of Science, Technology and Medicine, University of Luxemburg, Esch-Belval, Luxemburg

[9] Vrije Universiteit Brussel (VUB), Research Group GRON, Brussels, Belgium

Corresponding author: becker.marianne@chl.lu

Abstract

Introduction Familial hypercholesterolaemia (FH) is a frequent (1:250) autosomal dominantly inherited condition which causes precocious cerebral-cardio vascular disease (CVD) in early adulthood. Early detection and initiation of treatment can prevent the development of CVD and premature death. Our pilot study aims to investigate the prevalence of familial hypercholesterolaemia, the feasibility and efficacy of a screening based on a capillary blood test performed during a school medicine visit in primary school children. An adjacent cascade screening targets to detect other affected family members.

Methods and analysis In this cross-sectional study all children (n=3200) between 7-12 years, attending primary school in the city of Luxemburg, and invited for their mandatory medical school exam between 2021 and 2023, are invited to participate.

Families receive written information with an informed consent form. Children, who come to the medical visit with the signed informed consent form, are included. The study nurse performs a capillary blood test. Families receive the result including an interpretation and invitation to seek medical advice if indicated. If familial hypercholesterolaemia is confirmed, a cascade screening in that family will be proposed. The child will receive standard care.

Primary outcome is the occurrence of confirmed familial hypercholesterolaemia in the study population. Secondary outcomes include the percentage of children screened, percentage of children with abnormal lipid values, percentage of families screened and percentage of families with additionally identified members suffering from hypercholesterolaemia. A health economic analysis will be performed.

Ethics and dissemination: Ethics approval has been obtained from the National Research Ethics Committee (CNER (Luxemburg)) and was authorized by the ministry of health in Luxemburg. The results will be disseminated in peer-reviewed publications, conference presentations and by public media to the general public.

Trial registration number: NCT05271305; clinicaltrials.gov.

Strengths of this study

- **All school aged children in the city of Luxemburg can be tested.**
- **Minimal invasive sampling (capillary blood test) is used.**
- **The screened population is young enough to prevent cardiovascular disease if FH is diagnosed and treated in these children.**
- **Affected family members may be identified via adjacent cascade screening before cardio-cerebral-vascular events.**
- **Health economic analysis will provide insight in the cost/benefit of a nation-wide screening.**

Limitations of this study

- **Opt-in approach and recruitment may limit the participation rate.**

Introduction

Familial hypercholesterolaemia (FH) is an autosomal dominantly inherited genetic disorder, which causes premature arteriosclerosis leading to cardio- and cerebrovascular disease. FH is frequent with an estimated prevalence of 1:217[1] to 1:250[2]. Mutations are often found in the low-density lipoprotein receptor gene (LDLr), apolipoprotein B gene (APOB), or the proprotein convertase subtilisin/kexin type 9 gene (PCSK9).

Most of the affected persons are not aware of their condition. A recent publication estimated, that only 10% of affected patients are diagnosed and treated [3]. Vascular pathology develops silently and often FH is not recognised before the first –potentially fatal- heart attack or stroke at a young age (before 40 years). Patients suffering from FH have a 100 fold higher risk to die from a coronary heart disease at the age of 20-39 years[4].

As this development can be avoided by an early diagnosis and treatment with cholesterol lowering medication started in childhood[5,6], FH is a suitable candidate for screening[7]. The cholesterol lowering therapy is available for children from 6 years onwards[8]. A study in England showed that FH screening based on capillary blood tests in 1-2 year old toddlers is cost-effective[9]. At the Technical Meeting of 2021 Slovenian EU presidency, broad professional consensus on paediatric FH screening were presented and public policy recommendations were developed[10]. Despite these facts, so far Slovenia is the only country with a national universal screening program[11].

In Luxemburg – as in many other developed countries- cardiovascular diseases are the leading cause of death[12,13]. Preventing cardiovascular disease will hence not only improve and save lives of affected individuals, but will as well lower the financial burden for the national health care systems: The cost of an universal screening per diagnosed case has been estimated at 2.500 € by Wald et al.[9]. The Luxemburg Institute of health (LIH) estimated in 2016 the cost of a myocardial infarction survivor in Luxemburg at 15,200 € in the first year and at 2,900 € for every following year. The cost for a cerebrovascular event survivor in Luxemburg was estimated at 19,500 € in the first year and 7,200 € for every following year.

We hypothesize that a screening based on capillary blood tests in the setting of the medical school visit in primary school children will be able to detect affected children and by applying the cascade screening we expect to identify affected family members.

We will assess the acceptance of this screening and provide further insight in the cost-effectiveness of this screening approach.

Methods and Analysis

Overview

Cross sectional design, targeting all primary school children (grade 2-6) in the city of Luxemburg. The study will be performed during the mandatory medical school exam (see figure 1) during the school years 2021/2022 and 2022/2023. If indicated, further medical follow-up is offered in the National Paediatric Clinic (Diabetes & Endocrine Care Clinic for Pediatric patients, DECCP). The creation of data collection tools and storage as well as the statistical analysis will be delivered by LIH Competence Centre for Methodology and Statistics.

STROBE reporting guidelines were applied in the preparation of this article [14].

Inclusion criteria

- Children, aged 7-12 years, attending primary school classes of the 2nd to 6th grade in Luxemburg City in 2021/2022 and 2022/2023 and who are invited for the medical school exam.
- Written informed consent of the parents/caregivers

Exclusion criteria

- No or an incomplete written informed consent at the medical school visit.

Recruitment

Recruitment takes place during the school year 2021/2022 and 2022/2023. The families of children who are invited to the medical school exam, receive written information about the screening and an informed consent form.

This information material includes a flyer in 4 languages (see figure 2), a flyer for children adapted to their age (flyer for 7-8 years and flyer 9-12 years), detailed information about the study for the parents/caregivers.

They will receive as well a questionnaire on the family history of premature cardio-cerebrovascular events and known FH disease (see figure 3).

We will promote this study in order to achieve as high a participation rate as possible and in order to raise awareness for FH. National scientific societies of cardiology, neurology, paediatrics and general medicine support the study.

Patient and Public Involvement

Participants were not directly involved in the study design. But patients were involved in the dissemination of the study by giving an interview explaining the interest of our study and patients and public are a main target of the dissemination of our results in order to enhance awareness for FH.

Study Procedures

A dedicated study nurse will collect the signed informed consent forms and will perform the finger prick when the children have their medical school exam (see figure 1).

She will fill out the electronic Case Record Form (height, weight and blood pressure measurement, data on family history regarding hypercholesterolemia and precocious cardiovascular disease). All data (pseudonymised) on each participant will be entered in the online database (developed by the LIH by using the Vanderbilt REDcap system) for further analysis. Data will be expressed in age adjusted scores.

Lipid profile measurement

The capillary blood sample (15 µl) will be analysed by the Alere Afinion 2 Analyser, using the Alere Afinion Lipid Panel. Test result is available in 7 minutes and can be printed. The Alere Afinion Lipid Panel includes the analysis of total cholesterol, HDL and triglycerides by a colorimetric ELISA method. Based on these results Alere Afinion 2 Analyser will calculate LDL. A comparison between this handhold machine and CHL (Centre hospitalier de Luxembourg) laboratory method (Colorimetric, enzymatic assays, Roche Cobas 8000) had been conducted and demonstrated a good correlation between the Cholesterol, LDL, HDL and triglyceride measurements.

Information for the families

A letter with the result of the lipid test will be sent to the family. This will include the confirmation that the child has a normal result (total cholesterol (CT) < 200 mg/dl and LDL cholesterol (LDLC) < 130 mg/dl), or a recommendation to contact their doctor when the cholesterol level is slightly elevated (CT 200-230 mg/dl and/or LDLC 130-160 mg/dl). If cholesterol levels are high (CT > 230 mg/dl and/or LDLC > 160 mg/dl), the family will receive a letter with an invitation for a further clinical evaluation and the advice to contact their doctor or to make an appointment directly in the paediatric clinic.

Follow-up in case of high cholesterol levels

When a high cholesterol level is detected, a detailed family history together with fasting blood tests are required.

If the pathological values are confirmed, dietary counselling is indicated followed by a fasting blood control (including a genetic analysis for FH panel, lipoprotein(a), GOT, GPT, CK, TSH) 3 months later.

Familial hypercholesterolaemia is confirmed if:

- genetically confirmed + LDL cholesterol > 130 mg/dl
- no mutation, but 2x LDL cholesterol > 190 mg/dl
- no mutation, but 2x LDL cholesterol >160 mg/dl and precocious cardiovascular diseases in the family

If FH is confirmed,

- a cascade screening is offered to the first-degree family members (and if confirmed in those to the related second-degree family members too).
- cIMT measurement before treatment will be performed.
- children will be offered a treatment and adult family members will be offered a follow-up by adult lipid specialists
- paediatric patients will be offered a follow-up once every 6 months with control of the lipid results and adaption of their therapy.

Data management

A specific electronic CRF (eCRF) is developed for the study with items related to the socio-demographic characteristics of the participants and familial

hypercholesterolaemia. CRF data will be entered online in the eCRF and the data will be stored in a secured data base.

The database has been designed and the eCRF developed by the Competence Center for Methodology and Statistics of LIH with a GDPR compliant data management system. LIH will be responsible for the data quality control, cleaning and data analysis.

Real time Z-scores for height, BMI and blood pressure for each participant are made available on the e-CRF

For Z-score calculation of height and BMI, L, M and S values are taken from the WHO 2007 growth reference data for children and adolescents (*https://www.who.int/tools/growth-reference-data-for-5to19-years/indicators*).

For Z-scores of systolic and diastolic blood pressure, NHANES reference for the height L, M and S values are used.

Out of range alerts are available in the e-CRF for total cholesterol and LDL cholesterol values. The alerts confirm if the values for the participant are normal, slightly abnormal or abnormal. Specific validation checks are created in e-CRF for discrepancies between normal and abnormal laboratory values and the follow up action for the families. For example, if total cholesterol and LDL values are abnormal, but the follow-up action is selected as "*Normal Result- Result transmitted to family*", a query would pop up, alerting this discrepancy

All personal and clinical data will be pseudonymised with an ID-number (e.g. IF0001) accessible only to the dedicated employees. The delegation list of all clinical team members will be kept at the DECCP and must be signed by the principal investigator. All access and changes in the data is tracked and monitored in an audit trail. Therefore, data collection, storage and in-depth analysis respect the highest standards of data protection and security.

Only pseudonymised data without any link to personal data will be accessible by the dedicated members of LIH and collaborating members and partners for analysis and further investigation.

Statistical methods

Sample size calculation

The primary outcome is the prevalence of familial hypercholesterolaemia in children between 7-12 years in Luxemburg. When the sample size is 1501, a two-sided 95% confidence interval for a single proportion using the large sample normal approximation adjusted for a finite population of size 39,000 (children of 7-12 years in Luxemburg) will extend 0,00313 from the observed proportion for an expected proportion of 0,005 of familial hypercholesterolaemia.

Sampling plan

The sampling plan will be stratified and randomized with an allocation probability (chance to be selected is equal for all individuals of the same age category and gender) proportional to size (of the population) without replacement (the same individual could not be selected twice). The probability to be sampled nhZhi will be calculated for each individual based on the size of each age and gender strata in the sample. It will be included as a weighting parameter in the calculation of the prevalence Σ(xi_nhZhi), which will be estimated by summing up data on all patients.

Statistical analyses

A statistical analysis plan detailing the statistical analysis will be written blinded to the data and before the end of the enrolment.

A check for missing data will be performed. Several methods for processing missing data are possible depending on the type of missing responses (missing completely at random, missing at random or not missing at random).

The primary outcome, prevalence of familial hypercholesterolaemia in children between 7-12 years in Luxemburg, will be calculated by summing up data on all patients and weighting by the size of each age and gender strata as described earlier.

To include a finite population correction in Taylor series variance estimation, the size of each age and gender strata in the target population will also be entered in the analyses in order to calculate the Wald 95% confidence interval, and thereby extrapolate the proportion of familial hypercholesterolaemia to the target population. The surveyfreq procedure of the statistical software SAS System V9.3 (SAS Institute, Cary, NC) will be used.

Secondary outcomes be calculated in a similar way as the primary outcome if possible in order to enable inference to the target population of children between 7-12 years in Luxemburg.

The secondary outcomes are:

- Percentage of children screened (number screened/number invited)
- Percentage of children with abnormal values
- Percentage of children with confirmed hypercholesterolaemia and treatment
- Percentage of families screened
- Percentage of families with additional family members with confirmed hypercholesterolaemia and treatment

Ethics and Dissemination

This study has received approval from the National Research Ethics Committee in Luxemburg (202108/01) and the Ministry of Health.

All parents/guardians will be provided with detailed written information about the study procedure. Written informed consent is necessary in order to include a child in this study. All results will be published anonymously to ensure that no participant can be identified. The name and identity of the participants will not appear in any of the published materials.

The results of this study will be disseminated in medical and scientific pre-reviewed publications and conference presentations.

Footnotes

- **Contributors:** MB and CdB conceived the study. MB, CdB, KW, DWD, SH, FF, BZ initiated the study design and AA helped with the implementation. MB and CdB are grant holders. MV, PM and VB provided statistical expertise in clinical trial design. All authors contributed to refinement of the study protocol and approved the final manuscript.

- **Acknowledgement:** We thank Michael Witsch who has helped to set up the z-score calculation for height, bmi and blood pressure.
- **Funding statement:** This work was supported by the non-profit organization "Coeur- Daniel Wagner", grant number: N/A.
- **Competing interests statement.**

  MB and DWD participated in a scientific advisory board of Daiichi Sankyo.

  AA, SH FF, MV, KW, BZ, SH, PM, VC and CdB have nothing to declare.

References

1 Benn M, Watts GF, Tybjærg-Hansen A, *et al.* Mutations causative of familial hypercholesterolaemia: screening of 98 098 individuals from the Copenhagen General Population Study estimated a prevalence of 1 in 217. *Eur Heart J* 2016;**37**:1384–94. doi:10.1093/eurheartj/ehw028

2 Wald DS, Wald NJ. Integration of child–parent screening and cascade testing for familial hypercholesterolaemia. *J Med Screen* 2019;**26**:71–5. doi:10.1177/0969141318796856

3 Representatives of the Global Familial Hypercholesterolemia Community. Reducing the Clinical and Public Health Burden of Familial Hypercholesterolemia: A Global Call to Action. *JAMA Cardiology* 2020;**5**:217–29. doi:10.1001/jamacardio.2019.5173

4 Risk of fatal coronary heart disease in familial hypercholesterolaemia. Scientific Steering Committee on behalf of the Simon Broome Register Group. *BMJ* 1991;**303**:893–6.

5 Luirink IK, Wiegman A, Kusters DM, *et al.* 20-Year Follow-up of Statins in Children with Familial Hypercholesterolemia. *N Engl J Med* 2019;**381**:1547–56. doi:10.1056/NEJMoa1816454

6 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. doi:10.1093/eurheartj/ehv157

7 Kusters DM, de Beaufort C, Widhalm K, *et al.* Paediatric screening for hypercholesterolaemia in Europe. *Arch Dis Child* 2012;**97**:272–6. doi:10.1136/archdischild-2011-300081

8 Vuorio A, Kuoppala J, Kovanen PT, *et al.* Statins for children with familial hypercholesterolemia. *Cochrane Database Syst Rev* 2017;**7**:CD006401. doi:10.1002/14651858.CD006401.pub4

9 Wald DS, Bestwick JP, Morris JK, *et al.* Child-Parent Familial Hypercholesterolemia Screening in Primary Care. *N Engl J Med* 2016;**375**:1628–37. doi:10.1056/NEJMoa1602777

10 Groselj U, Wiegman A, Gidding SS. Screening in children for familial hypercholesterolaemia: start now. *European Heart Journal* 2022;:ehac224. doi:10.1093/eurheartj/ehac224

11 Groselj 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. doi:10.1016/j.atherosclerosis.2018.06.858

12 Scharel L. Statistiques des causes de décès pour l'année 2019. ;:84.

13 Global Health Estimates: Life expectancy and leading causes of death and disability. https://www.who.int/data/gho/data/themes/mortality-and-global-health-estimates (accessed 26 Feb 2022).

14 von Elm E, Altman DG, Egger M, *et al.* The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement: guidelines for reporting observational studies. *Lancet* 2007;**370**:1453–7. doi:10.1016/S0140-6736(07)61602-X

- **Word Count:** 2131 words.

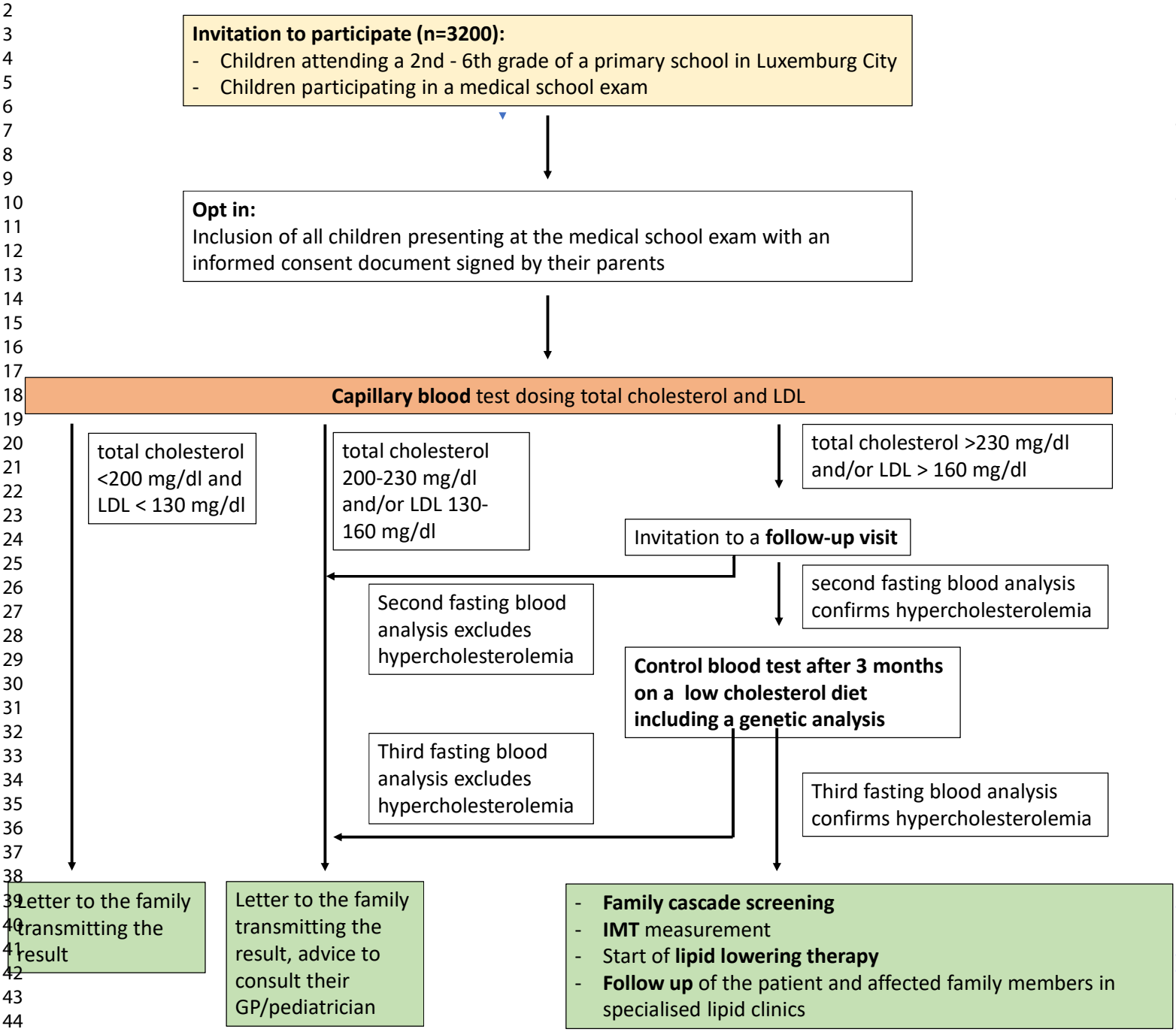

Figure 1 Study flowchart. IMT, intima media thickness

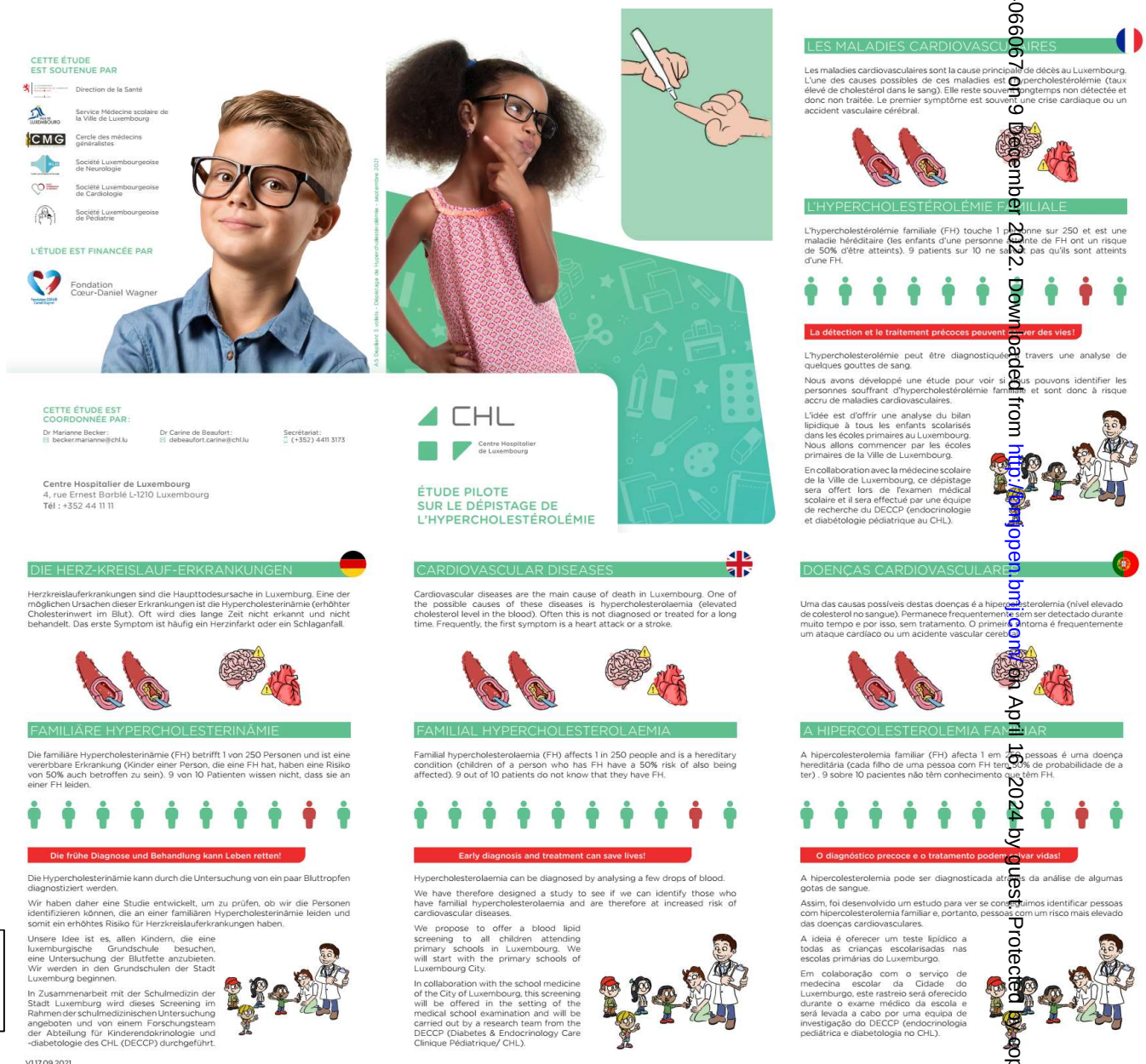

Figure 2
Information material: Flyer in 4 languages

Questionnaire for parents in the setting of the hypercholesterolemia screening study:

- Do you have an elevated cholesterol level?
  Father: ☐ Yes ☐ No ☐ I do not know my cholesterol level.
  If yes: Are you receiving medication for it? ☐ Yes ☐ No

  Mother: ☐ Yes ☐ No ☐ I do not know my cholesterol level.
  If yes: Are you receiving medication for it? ☐ Yes ☐ No

- Does someone in your family suffer or suffered from a cardiovascular disease (heart attack, stroke) already at a young age (women < 60 years, men < 55 years)?
  ☐ Yes ☐ No
  - If yes, who? _____

Figure 3: English part of the questionnaire for parents

STROBE Statement—Checklist of items that should be included in reports of ***cross-sectional studies***

	Item No	Recommendation	Page No
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	1
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	2-3
Objectives	3	State specific objectives, including any prespecified hypotheses	3
Methods			
Study design	4	Present key elements of study design early in the paper	4
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	4-7
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants	4
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	8,9
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	*5-7*
Bias	9	Describe any efforts to address potential sources of bias	8
Study size	10	Explain how the study size was arrived at	7,8
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	6,7
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	8
		(*b*) Describe any methods used to examine subgroups and interactions	n/a
		(*c*) Explain how missing data were addressed	8
		(*d*) If applicable, describe analytical methods taking account of sampling strategy	8
		(*e*) Describe any sensitivity analyses	8
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	n/a
		(b) Give reasons for non-participation at each stage	n/a
		(c) Consider use of a flow diagram	Figure 1
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	n/a
		(b) Indicate number of participants with missing data for each variable of interest	n/a
Outcome data	15*	Report numbers of outcome events or summary measures	n/a

Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	n/a
		(*b*) Report category boundaries when continuous variables were categorized	n/a
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	n/a
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	n/a
Discussion			
Key results	18	Summarise key results with reference to study objectives	n/a
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	n/a
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	n/a
Generalisability	21	Discuss the generalisability (external validity) of the study results	n/a
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	10

*Give information separately for exposed and unexposed groups.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

BMJ Open

Screening for familial hypercholesterolaemia in primary school children: protocol for a cross-sectional, feasibility study in Luxemburg city (EARLIE)

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2022-066067.R1
Article Type:	Protocol
Date Submitted by the Author:	09-Oct-2022
Complete List of Authors:	Becker, Marianne; Centre Hospitalier de Luxembourg, Pediatric endocrinology and diabetology (DECCP); VUB University, Research Group GRON Adamski, Aurélie; Centre Hospitalier de Luxembourg, Pediatric endocrinology and diabetology (DECCP) Fandel, Françoise; City of Luxembourg, Department of School Medicine Vaillant, Michel; Luxembourg Institute of Health, Competence Centre for Methodology and Statistics Wagner, Kerstin; Centre Hospitalier de Luxembourg, Department of paediatric cardiology Droste, Dirk; Centre Hospitalier de Luxembourg, Department of Neurology Ziade, Bechara; Luxembourg Ministry of Health, Direction Hein, Steve; Centre Hospitalier de Luxembourg, Department of sports medicine Mendon, Priyanka; Luxembourg Institute of Health, Competence Center for Methodology and Statistics Bocquet, Valéry; Luxembourg Institute of Health, Competence Center for Methodology and Statistics de Beaufort, Carine; Centre Hospitalier de Luxembourg, Department of paediatric endocrinology and diabetology (DECCP); University of Luxembourg - Belval Campus, Faculty of Science, Technology and Medicine
Primary Subject Heading:	Paediatrics
Secondary Subject Heading:	Cardiovascular medicine, Diagnostics, Epidemiology, Health economics
Keywords:	Lipid disorders < DIABETES & ENDOCRINOLOGY, Paediatric endocrinology < DIABETES & ENDOCRINOLOGY, Community child health < PAEDIATRICS, PUBLIC HEALTH, VASCULAR MEDICINE

Screening for familial hypercholesterolaemia in primary school children: protocol for a cross-sectional, feasibility study in Luxemburg city (EARLIE)

Marianne Becker[1,9], Aurélie Adamski[1], Françoise Fandel[2], Michel Vaillant[3], Kerstin Wagner[4], Dirk W. Droste[5], Bechara Ziade[6], Steve Hein[7], Priyanka Mendon[3], Valéry Bocquet[3], Carine de Beaufort[1,8]

[1] Department of paediatric endocrinology and diabetology (DECCP), Centre hospitalier de Luxembourg, Luxembourg
[2] Department of School Medicine, City of Luxembourg, Luxembourg
[3] Competence Center for Methodology and Statistics, Luxembourg Institute of Health, Luxembourg
[4] Department of paediatric cardiology, Centre hospitalier de Luxembourg, Luxembourg
[5] Department of Neurology, Centre Hospitalier de Luxembourg, Luxembourg
[6] Direction of the ministry of Health, Luxembourg
[7] Department of sports medicine, Centre hospitalier de Luxembourg, Luxembourg
[8] Faculty of Science, Technology and Medicine, University of Luxemburg, Esch-Belval, Luxembourg
[9] Vrije Universiteit Brussel (VUB), Research Group GRON, Brussels, Belgium

Corresponding author: becker.marianne@chl.lu

Abstract

Introduction Familial hypercholesterolaemia (FH) is a frequent (1:300) autosomal dominantly inherited condition which causes premature (female < 60 years, male < 55 years) cardio-cerebrovascular disease (CVD). Early detection and initiation of treatment can prevent the development of CVD and premature death. Our pilot study aims to investigate the prevalence of FH, the feasibility and efficacy of a screening based on a capillary blood test performed during a school medicine visit in primary school children.

Methods and analysis In this cross-sectional study all children (n=3200) between 7-12 years, attending primary school in the city of Luxemburg, and invited for their mandatory medical school exam between 2021 and 2023, are invited to participate. A study nurse performs a capillary blood test to analyse the lipid profile. Families receive

the result including an interpretation and invitation to seek medical advice if indicated. If FH is confirmed, a reverse cascade screening in that family will be proposed. The child will receive standard care. Primary outcome is the occurrence of confirmed FH in the study population. Secondary outcomes include the percentage of children screened, percentage of children with abnormal lipid values, percentage of families screened and percentage of families with additionally identified members suffering from hypercholesterolaemia. A health economic analysis will be performed.

Ethics and dissemination: Ethics approval (reference number 202108/01) has been obtained from the National Research Ethics Committee (CNER (Luxemburg)) and was authorized by the ministry of health in Luxemburg. Families receive written information with an informed consent form. Participation requires an informed consent form signed by the parents. The results will be disseminated in peer-reviewed publications, conference presentations and by public media to the general public.

Study registration number: NCT05271305; clinicaltrials.gov.

Strengths and limitations of this study

- **All school aged children in the city of Luxemburg can be tested with minimal invasive sampling (capillary blood test).**
- **The young age at screening allows prevention of cardiovascular disease in those who test positive for FH and detection and treatment of affected family members via reverse cascade screening before cardio-cerebrovascular events.**
- **Health economic analysis will provide insight in the cost/benefit of a nation-wide screening.**
- **Opt-in approach and recruitment may limit the participation rate and referral to specialist care after detection might not be realised due to underestimation of FH by some paediatricians and family doctors**
- **Confirmation by fasting venous blood samples might decrease the participation rate.**

Introduction

FH is an autosomal dominantly inherited genetic disorder, which causes premature arteriosclerosis leading to cardio- and cerebrovascular disease[1,2]. FH is frequent with an estimated prevalence of 1:276[3] to 1:310[4,5]. Mutations are often found in the low-density lipoprotein receptor gene (*LDLR*), apolipoprotein B gene (*APOB*), or the proprotein convertase subtilisin/kexin type 9 gene (*PCSK9*)[6].

Most of the affected persons are not aware of their condition. A recent publication estimated, that only 10% of affected patients are diagnosed and treated[7]. Vascular pathology develops silently and often FH is not recognised before the first –potentially fatal- heart attack or stroke at a young age (before 40 years). Patients suffering from FH have a standardized incidence ratio to develop a coronary heart disease between the age of 25 and 40 years of 11.1 for men and 17.3 for women[8].

As this development can be avoided by an early diagnosis and treatment with cholesterol lowering medication started in childhood[9,10], FH is a suitable candidate for screening[11]. The cholesterol lowering therapy is available for children from 6 years onwards[12]. A study in England showed that FH screening based on capillary blood tests in 1-2 year old toddlers is cost-effective[13]. At the Technical Meeting of 2021 Slovenian EU presidency, broad professional consensus on paediatric FH screening were presented and public policy recommendations were developed[14]. Despite these facts, so far Slovenia is the only country with a national universal screening program[15].

An opt-out approach – as applied in the Slovenian screening program- would very likely result in a higher participation rate[16], but due to ethical restrictions, only an opt-in approach was feasible for this study.

In Luxemburg – as in many other developed countries- cardiovascular diseases are the leading cause of death[17,18]. Preventing cardiovascular disease will hence not only improve and save lives of affected individuals, but will as well lower the financial burden for the national health care systems: The cost of an universal screening per diagnosed case has been estimated at 2.500 € by Wald et al.[13]. The Luxemburg Institute of health (LIH) estimated in 2016 the cost of a myocardial infarction survivor in Luxemburg at 15,200 € in the first year and at 2,900 € for every following year. The

cost for a cerebrovascular event survivor in Luxemburg was estimated at 19,500 € in the first year and 7,200 € for every following year.

We hypothesize that a screening based on capillary blood tests in the setting of the medical school visit in primary school children will be able to detect affected children and by applying a reverse cascade screening we expect to identify affected family members. We will assess the acceptance of this screening and provide further insight in the cost-effectiveness of this screening approach.

Methods and Analysis

Overview

Cross sectional design, targeting all primary school children (grade 2-6) in the city of Luxemburg. The study will be performed during the mandatory medical school exam (see figure 1). If indicated, further medical follow-up is offered in the National Paediatric Clinic (Diabetes & Endocrine Care Clinic for Pediatric patients, DECCP). The creation of data collection tools and storage as well as the statistical analysis will be delivered by LIH Competence Centre for Methodology and Statistics.

STROBE reporting guidelines were applied in the preparation of this article [19].

Inclusion criteria

- Children, aged 7-12 years, attending primary school classes of the 2nd to 6th grade in Luxemburg City in 2021/2022 and 2022/2023 and who are invited for the medical school exam.
- Written informed consent of the parents/caregivers

Exclusion criteria

- No or an incomplete written informed consent at the medical school visit.

Recruitment

In primary schools, medical school examinations take place every 2 years. The families of children who are invited to the medical school exam, receive written information

about the screening and an informed consent form. As the study is running over a period of 2 years, every child will have been invited by the end of the recruitment period.

This information material includes a flyer in 4 languages (see figure 2), a flyer for children adapted to their age (flyer for 7-8 years and flyer 9-12 years), and detailed information about the study for the parents/caregivers.

They will receive as well a questionnaire on the family history of premature cardio-cerebrovascular events and known FH disease (see figure 3).

We will promote this study in order to achieve as high a participation rate as possible and in order to raise awareness for FH. Promotion will include interviews in the lay press, distribution of information material to paediatricians and general practitioners, teachers and the parents' representative committee. National scientific societies of cardiology, neurology, paediatrics and general medicine support the study.

Patient and Public Involvement

The study is in line with the demand of FH patients' groups (FH Europe), to implement a paediatric screening for FH in Europe (https://fheurope.org/policy/prague-declaration/). In the design of the protocol there was no patient involvement, but there is patient involvement and support in the promotion of the study. Dissemination of the study results is planned in scientific journals but equally in lay media in order to enhance awareness of FH.

Study Procedures

A dedicated study nurse will collect the signed informed consent forms and will perform a finger prick using a Medlance® plus special blade (0,8 mm) lancet when the children have their medical school exam (see figure 1).

The nurse will fill out the case record form (CRF, see supplemental material 1), documenting height, weight and blood pressure measurement, data on family history regarding hypercholesterolemia and precocious cardiovascular disease. All data (pseudonymised) will be entered in the online database (developed by the LIH by using the Vanderbilt REDcap system) for further analysis. Data will be expressed in age adjusted scores.

Lipid profile measurement

The capillary blood sample (15 µl) will be analysed by the Alere Afinion 2 Analyser, using the Alere Afinion™ Lipid Panel. Test result is available in 7 minutes and can be printed. The Alere Afinion™ Lipid Panel includes the analysis of total cholesterol (TC), high-density lipoprotein (HDL) and triglycerides by a colorimetric ELISA method. Based on these results Alere Afinion 2 Analyser will calculate low-density lipoprotein (LDL). A comparison between this handhold machine and CHL (Centre hospitalier de Luxembourg) laboratory method (Colorimetric, enzymatic assays, Roche Cobas 8000) had been conducted and demonstrated a good correlation between the TC, LDL, HDL and triglyceride measurements.

Information for the families

A letter with the result of the lipid test will be sent to the family. This will include the confirmation that the child has a normal result (TC < 200 mg/dl and LDL < 130 mg/dl), or a recommendation to contact their doctor when the cholesterol level is slightly elevated (TC 200-230 mg/dl and/or LDL 130-160 mg/dl). If cholesterol levels are high (TC > 230 mg/dl and/or LDL > 160 mg/dl), the family will receive a letter with an invitation for a further clinical evaluation and the advice to contact their doctor or to make an appointment directly in the paediatric clinic.

As this first blood test is performed non-fasting, applying a calculation of the LDL levels according to Friedewald, high triglyceride levels could lead to falsely elevated LDL levels.

Follow-up in case of high cholesterol levels

When a high cholesterol level is detected, a detailed family history together with a fasting blood test (fast for at least 8 hours) are required.

If the pathological values are confirmed, dietary counselling is indicated followed by a fasting blood control (including TC, LDL, HDL, a genetic analysis for FH panel, lipoprotein(a), serum glutamic oxaloacetic transaminase (GOT), serum glutamic pyruvic transaminase (GPT), creatine kinase (CK), thyroid-stimulating hormone (TSH)) 3 months later.

Genetic analysis is coordinated and results are interpreted by the genetic department of the Luxemburgish national health laboratory (Laboratoire national de santé, LNS). FH panel is performed at CHU Liège and includes the following genes: *LDLR* (NM_000527.4), *APOB* (NM_000384.2), *PGSK9* (NM_174936.3), *APOE* (NM-a00041.3), *LDLRAP1* (NM_015627.2), *LIPA* (NM_000235.3), *ABCG5* (NM_022436.2), *ABCG8* (NM_022437.2) and *STAP1* (NM_012108.3).

FH is confirmed if[9]:

- genetically confirmed + LDL > 130 mg/dl
- no mutation, but 2x LDL > 190 mg/dl
- no mutation, but 2x LDL >160 mg/dl and precocious cardiovascular diseases in the family

If FH is confirmed,

- cascade screening is offered to the first-degree family members (and if confirmed in those to the related second-degree family members too).
- carotid intima-media thickness (cIMT) measurement before treatment will be performed.
- children will be offered treatment and adult family members will be offered follow-up by adult lipid specialists
- paediatric patients will be offered follow-up once every 6 months with control of the lipid results and adaption of their therapy.

As lipoprotein (a) is an inherited causal risk factor for the development of cardiovascular disease[20], elevated lipoprotein (a) levels (> 50 mg/dl) will guide us to a more aggressive lipid lowering therapy in confirmed FH cases and to initiate a lipid-lowering therapy in borderline cases[21].

cIMT measures will be performed by the same investigator with a Siemens Acuson S2000 device using a 4 to 9 MHz linear probe. Diastolic far-wall common carotid intima-media thickness will be assessed proximally to the bifurcation where the vessel wall is parallel, using the cursors of the software and following the leading edge system[22].

If indicated, children will be treated with statins, evt. if older than 10 years and insufficient decrease of LDL under statin therapy is achieved, ezetimibe might be added.

If discordant fasting cholesterol levels are obtained (in the two fasting tests) in combination with a negative family history and no genetic mutation, the patient will be offered further follow up including a repeat blood test after several months.

In case of a proven genetic mutation, genetic analysis will be offered to other affected family members.

Data management

A specific electronic CRF (eCRF) is developed for the study with items related to the socio-demographic characteristics of the participants and FH. CRF data will be entered online in the eCRF and the data will be stored in a secured data base.

The database has been designed and the eCRF developed by the Competence Center for Methodology and Statistics of LIH with a GDPR compliant data management system. LIH will be responsible for the data quality control, cleaning and data analysis.

Real time Z-scores for height, BMI and blood pressure for each participant are made available on the e-CRF

For Z-score calculation of height and BMI, L, M and S values are taken from the WHO 2007 growth reference data for children and adolescents (*https://www.who.int/tools/growth-reference-data-for-5to19-years/indicators*).

For Z-scores of systolic and diastolic blood pressure, NHANES reference for the height L, M and S values are used.

All personal and clinical data will be pseudonymised with an ID-number (e.g. IF0001) accessible only to the dedicated employees. The delegation list of all clinical team members will be kept at the DECCP and must be signed by the principal investigator. All access and changes in the data is tracked and monitored in an audit trail. Therefore, data collection, storage and in-depth analysis respect the highest standards of data protection and security.

Only pseudonymised data without any link to personal data will be accessible by the dedicated members of LIH and collaborating members and partners for analysis and further investigation.

Statistical methods

Sample size calculation

The primary outcome is the prevalence of FH in children between 7-12 years in Luxemburg. The sample size was calculated according to Machin and Campbell[23] based on several indicators i.e. the confidence level, 1 or 2 sided interval, the expected proportion, the aimed precision of the estimated proportion and the population size (from which a simple random sample will be taken without replacement). As a consequence, a two-sided 95% confidence interval for a single proportion using the large sample normal approximation adjusted for a finite population of size of 39,000 (children of 7-12 years in Luxemburg) and a precision of 0.0033 for an expected proportion of 0.005 of FH lead to a sample size equal to 1501.

Sampling plan

The sampling plan will be stratified and randomized with an allocation probability (chance to be selected is equal for all individuals of the same age category and gender) proportional to size (of the population) without replacement (the same individual could not be selected twice). The probability to be sampled nhZhi (nh is the sample size for stratum h, and Zhi is the relative size of unit i in stratum h) will be calculated for each individual based on the size of each age and gender strata in the sample. It will be included as a weighting parameter in the calculation of the prevalence Σ(xi_nhZhi), which will be estimated by summing up data on all patients. These weighting estimations are elements to correct prevalence estimations in case of extreme strata (undersized or oversized stratum).

Statistical analyses

A statistical analysis plan detailing the statistical analysis will be written blinded to the data and before the end of the enrolment.

A check for missing data will be performed. Several methods for processing missing data are possible depending on the type of missing responses (missing completely at random, missing at random or not missing at random).

The primary outcome, prevalence of FH in children between 7-12 years in Luxemburg, will be calculated by summing up data on all patients and weighting by the size of each age and gender strata as described earlier.

To include a finite population correction in Taylor series variance estimation, the size of each age and gender strata in the target population will also be entered in the analyses in order to calculate the Wald 95% confidence interval, and thereby extrapolate the proportion of FH to the target population. The surveyfreq procedure of the statistical software SAS System V9.3 (SAS Institute, Cary, NC) will be used.

To complete corrections on prevalence provided by the weighting process, adjustments on age and sex are planned in order to estimate the prevalence. These adjustments will also take into account discrepancies between theoretical and real numbers for each stratum as well as non-responses issue.

Secondary outcomes be calculated in a similar way as the primary outcome, if possible, in order to enable inference to the target population of children between 7-12 years in Luxemburg.

The secondary outcomes are:

- Percentage of children screened (number screened/number invited)
- Percentage of children with abnormal values
- Percentage of children with confirmed hypercholesterolaemia and treatment
- Percentage of families screened
- Percentage of families with additional family members with confirmed hypercholesterolaemia and treatment

Ethics and Dissemination

This study has received approval from the National Research Ethics Committee in Luxemburg (202108/01) and the Ministry of Health.

All parents/guardians will be provided with detailed written information about the study procedure. Written informed consent is necessary in order to include a child in this study. All results will be published anonymously to ensure that no participant can be identified. The name and identity of the participants will not appear in any of the published materials.

The results of this study will be disseminated in medical and scientific pre-reviewed publications and conference presentations.

Footnotes

- **Contributors:** MB and CdB conceived the study. MB, CdB, KW, DWD, SH, FF, BZ initiated the study design and AA helped with the implementation. MB and CdB are grant holders. MV, PM and VB provided statistical expertise in clinical trial design. All authors contributed to refinement of the study protocol and approved the final manuscript.
- **Acknowledgement:** We thank Michael Witsch who has helped to set up the z-score calculation for height, bmi and blood pressure.
- **Funding statement:** This work was supported by the non-profit organization “Coeur- Daniel Wagner”, grant number: N/A.
- **Competing interests statement.**
  MB and DWD participated in a scientific advisory board of Daiichi Sankyo.
  AA, SH FF, MV, KW, BZ, SH, PM, VC and CdB have nothing to declare.

References

1 Austin MA, Hutter CM, Zimmern RL, *et al.* Familial hypercholesterolemia and coronary heart disease: a HuGE association review. *Am J Epidemiol* 2004;**160**:421–9. doi:10.1093/aje/kwh237

2 Ference BA, Ginsberg HN, Graham I, *et al.* Low-density lipoproteins cause atherosclerotic cardiovascular disease. 1. Evidence from genetic, epidemiologic, and clinical studies. A consensus statement from the European Atherosclerosis Society Consensus Panel. *European Heart Journal* 2017;**38**:2459–72. doi:10.1093/eurheartj/ehx144

3 Pang J, Martin AC, Mori TA, *et al.* Prevalence of Familial Hypercholesterolemia in Adolescents: Potential Value of Universal Screening? *J Pediatr* 2016;**170**:315–6. doi:10.1016/j.jpeds.2015.11.019

4 Hu P, Dharmayat KI, Stevens CAT, *et al.* Prevalence of Familial Hypercholesterolemia Among the General Population and Patients With Atherosclerotic Cardiovascular Disease. *Circulation* 2020;**141**:1742–59. doi:10.1161/CIRCULATIONAHA.119.044795

5 Beheshti SO, Madsen CM, Varbo A, *et al.* Worldwide Prevalence of Familial Hypercholesterolemia: Meta-Analyses of 11 Million Subjects. *Journal of the American College of Cardiology* 2020;**75**:2553–66. doi:10.1016/j.jacc.2020.03.057

6 Austin MA, Hutter CM, Zimmern RL, *et al.* Genetic causes of monogenic heterozygous familial hypercholesterolemia: a HuGE prevalence review. *Am J Epidemiol* 2004;**160**:407–20. doi:10.1093/aje/kwh236

7 Representatives of the Global Familial Hypercholesterolemia Community. Reducing the Clinical and Public Health Burden of Familial Hypercholesterolemia: A Global Call to Action. *JAMA Cardiology* 2020;**5**:217–29. doi:10.1001/jamacardio.2019.5173

8 Mundal LJ, Igland J, Veierød MB, *et al.* Impact of age on excess risk of coronary heart disease in patients with familial hypercholesterolaemia. *Heart* 2018;**104**:1600–7. doi:10.1136/heartjnl-2017-312706

9 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. doi:10.1093/eurheartj/ehv157

10 Luirink IK, Wiegman A, Kusters DM, *et al.* 20-Year Follow-up of Statins in Children with Familial Hypercholesterolemia. *N Engl J Med* 2019;**381**:1547–56. doi:10.1056/NEJMoa1816454

11 Kusters DM, de Beaufort C, Widhalm K, *et al.* Paediatric screening for hypercholesterolaemia in Europe. *Arch Dis Child* 2012;**97**:272–6. doi:10.1136/archdischild-2011-300081

12 Vuorio A, Kuoppala J, Kovanen PT, *et al.* Statins for children with familial hypercholesterolemia. *Cochrane Database Syst Rev* 2017;**7**:CD006401. doi:10.1002/14651858.CD006401.pub4

13 Wald DS, Bestwick JP, Morris JK, *et al.* Child-Parent Familial Hypercholesterolemia Screening in Primary Care. *N Engl J Med* 2016;**375**:1628–37. doi:10.1056/NEJMoa1602777

14 Groselj U, Wiegman A, Gidding SS. Screening in children for familial hypercholesterolaemia: start now. *European Heart Journal* 2022;:ehac224. doi:10.1093/eurheartj/ehac224

15 Groselj 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. doi:10.1016/j.atherosclerosis.2018.06.858

16 Sustar U, Kordonouri O, Mlinaric M, *et al.* Universal screening for familial hypercholesterolemia in 2 populations. *Genet Med* 2022;:S1098-3600(22)00819-X. doi:10.1016/j.gim.2022.06.010

17 Scharel L. Statistiques des causes de décès pour l'année 2019. ;:84.

18 Global Health Estimates: Life expectancy and leading causes of death and disability. https://www.who.int/data/gho/data/themes/mortality-and-global-health-estimates (accessed 26 Feb 2022).

19 von Elm E, Altman DG, Egger M, *et al.* The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement: guidelines for reporting observational studies. *Lancet* 2007;**370**:1453–7. doi:10.1016/S0140-6736(07)61602-X

20 Welsh P, Welsh C, Celis-Morales CA, *et al.* Lipoprotein(a) and cardiovascular disease: prediction, attributable risk fraction, and estimating benefits from novel interventions. *European Journal of Preventive Cardiology* 2022;**28**:1991–2000. doi:10.1093/eurjpc/zwaa063

21 Catapano AL, Daccord M, Damato E, *et al.* How should public health recommendations address Lp(a) measurement, a causative risk factor for cardiovascular disease (CVD)? *Atherosclerosis* 2022;**349**:136–43. doi:10.1016/j.atherosclerosis.2022.02.013

22 Droste DW, Schulte V, Schomacher G, *et al.* Comparison of Automatic Intima Media Thickness Measurement and Eye Balling. *Cerebrovasc Dis* 2010;**29**:307–8. doi:10.1159/000278697

23 Machin D, etc, Campbell MJ. *Statistical tables for the design of clinical trials*. Illustrated edition. Oxford England ; Boston : St Louis, Mo: : Blackwell Scientific Publications 1987.

- **Word Count:** 2898 words.

Figure legends:

- Figure 1: Study flowchart. TC: total cholesterol; LDL: low-density lipoprotein; precocious CVD: cardiovascular disease < 60 years in males/ < 55 years in females; IMT: intima media thickness; GP: general practitioner
- Figure 2: Information material: Flyer in 4 languages. © 2021 Centre hospitalier de Luxembourg.
- Figure 3: English part of the questionnaire for parents.

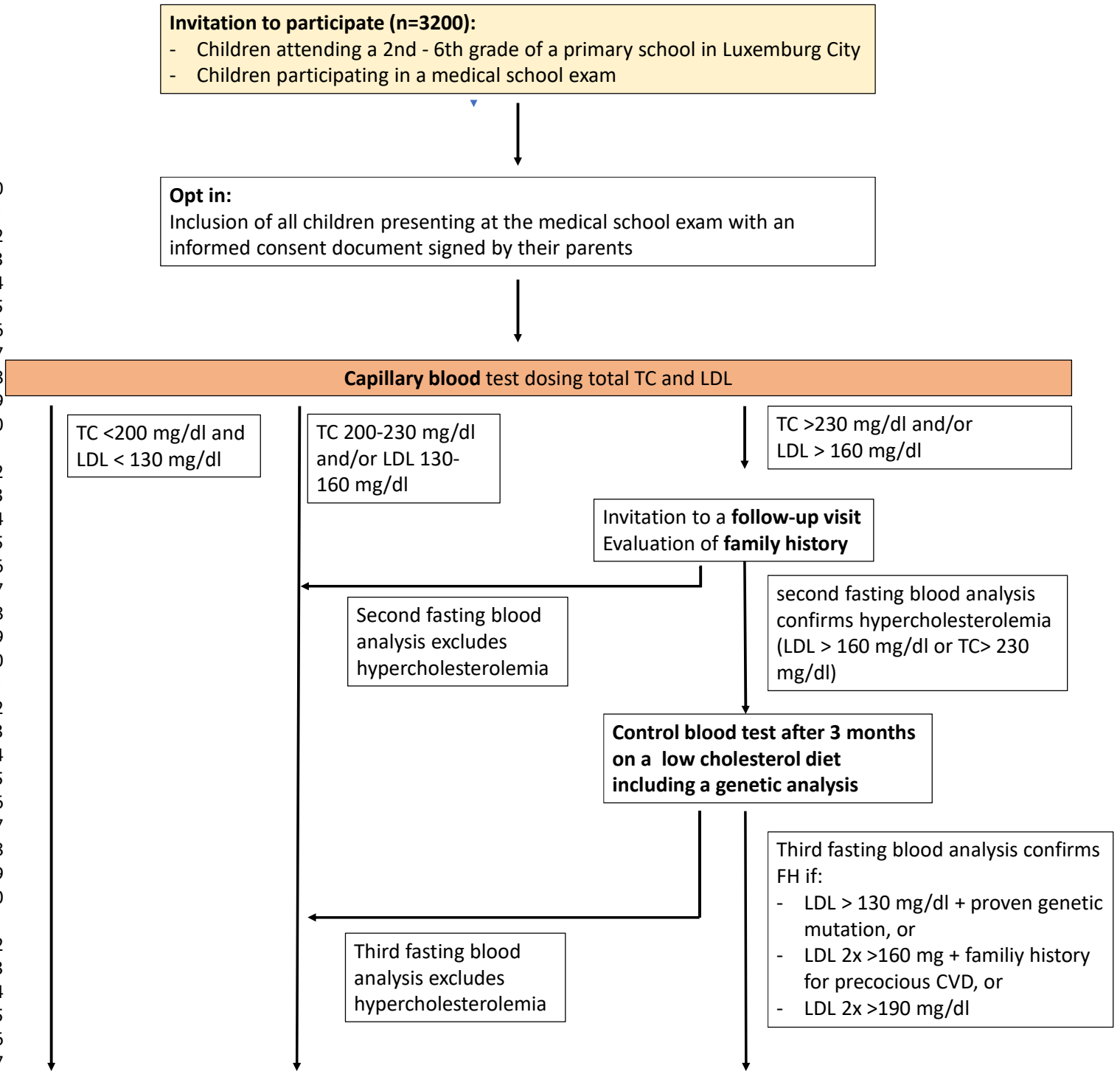

Figure 1 Study flowchart. TC: total cholesterol; LDL: low-density lipoprotein; precocious CVD: cardiovascular disease < 60 years in males/ < 55 years in females; IMT: intima media thickness; GP: general practicioner

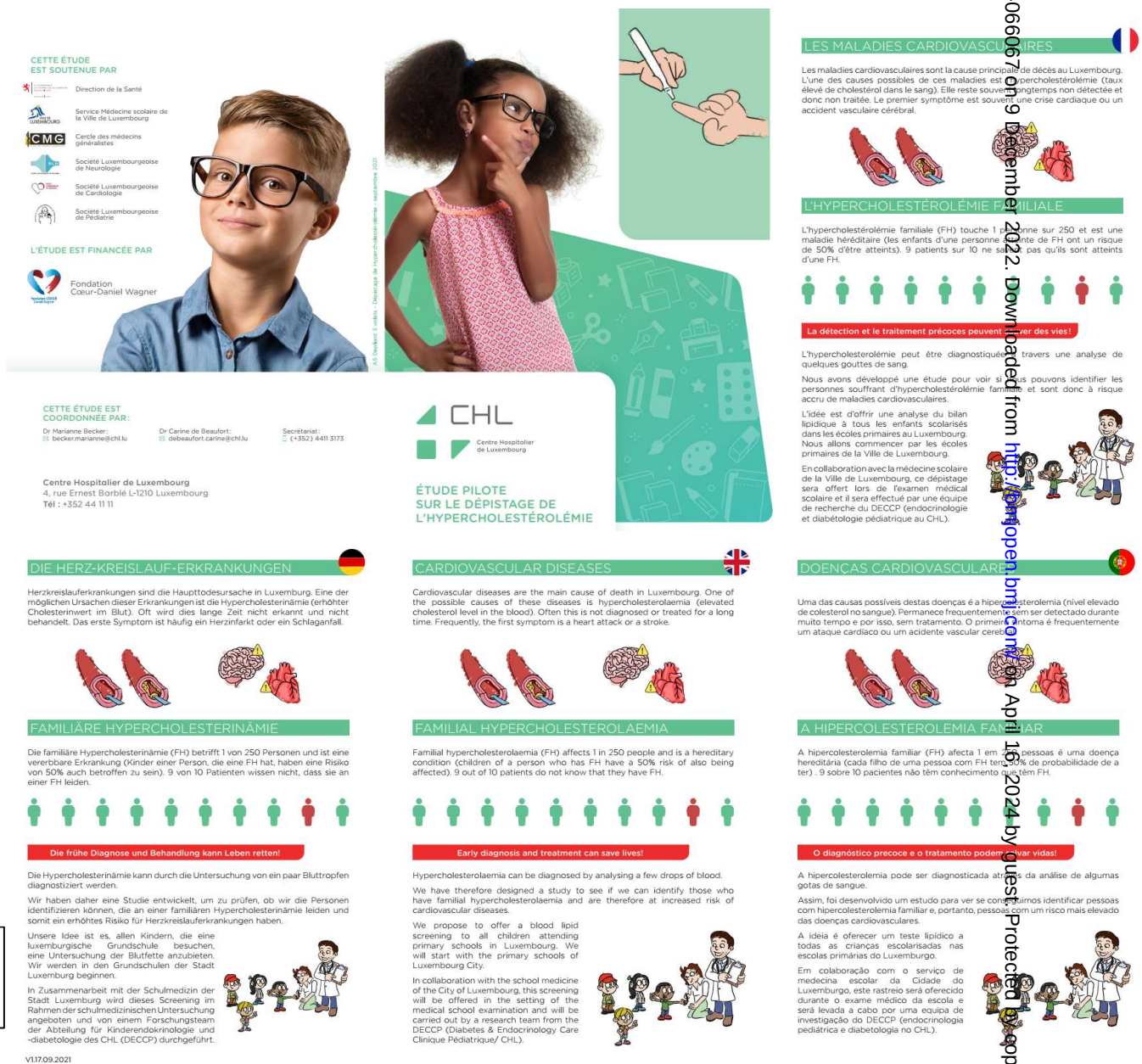

Figure 2
Information material: Flyer in 4 languages

Questionnaire for parents in the setting of the hypercholesterolemia screening study:

- Do you have an elevated cholesterol level?
  Father: ☐ Yes ☐ No ☐ I do not know my cholesterol level.
  If yes: Are you receiving medication for it? ☐ Yes ☐ No

  Mother: ☐ Yes ☐ No ☐ I do not know my cholesterol level.
  If yes: Are you receiving medication for it? ☐ Yes ☐ No

- Does someone in your family suffer or suffered from a cardiovascular disease (heart attack, stroke) already at a young age (women < 60 years, men < 55 years)?
  ☐ Yes ☐ No
  - If yes, who? _____

Figure 3: English part of the questionnaire for parents

Supplemental material 1: CRF

CRF

Case report form,			Patient number
	FH Pilot national depistage		
Date of Birth		month	year
gender		☐male	☐female
weight (kg)			
height (cm)			
blood pressure (mmHg)			
Family Questionnaire		☐ yes	☐ no
if yes: high cholesterol father:	☐ yes	☐ no	☐ unknown
if yes: high cholesterol mother:	☐ yes	☐ no	☐ unknown
if yes: precocious CVD:		☐ yes	☐ no
if precocious CVD: who*:			
blood sample		☐ yes	☐ no
Time of sampling	day	month	hour
LABORATORY OUTCOME			
	result		ref range
Total cholesterol (mg/dl)			< 200
LDL cholesterol (mg/dl)			< 130
HDL Cholesterol (mg/dl)			> 45
triglycerides (mg/dl)			< 130
Further action	☐ normal result -> result transmitted to family		
	☐ slightly elevated -> result transmitted to family + proposition to see GP		
	☐ abnormal result -> result transmitted to family + invitation DECCP/see GP		
* GMM = grand-mère maternelle, GMP = grand-père paternel, TM = tante maternelle; TP = tante paternel			
CM = cousin/e maternel(le), CP = cousin/e paternel(le)			

eCRF:

Out of range alerts are available in the e-CRF for TC and LDL values. The alerts confirm if the values for the participant are normal, slightly abnormal or abnormal. Specific validation checks are created in e-CRF for discrepancies between normal and abnormal laboratory values and the follow up action for the families. For example, if TC and LDL values are abnormal, but the follow-up action is selected as "*Normal Result- Result transmitted to family*", a query would pop up, alerting this discrepancy

STROBE Statement—Checklist of items that should be included in reports of ***cross-sectional studies***

	Item No	Recommendation	Page No
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	1
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	2-3
Objectives	3	State specific objectives, including any prespecified hypotheses	3
Methods			
Study design	4	Present key elements of study design early in the paper	4
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	4-7
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants	4
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	8,9
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	*5-7*
Bias	9	Describe any efforts to address potential sources of bias	8
Study size	10	Explain how the study size was arrived at	7,8
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	6,7
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	8
		(*b*) Describe any methods used to examine subgroups and interactions	n/a
		(*c*) Explain how missing data were addressed	8
		(*d*) If applicable, describe analytical methods taking account of sampling strategy	8
		(*e*) Describe any sensitivity analyses	8
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	n/a
		(b) Give reasons for non-participation at each stage	n/a
		(c) Consider use of a flow diagram	Figure 1
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	n/a
		(b) Indicate number of participants with missing data for each variable of interest	n/a
Outcome data	15*	Report numbers of outcome events or summary measures	n/a

Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	n/a
		(*b*) Report category boundaries when continuous variables were categorized	n/a
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	n/a
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	n/a
Discussion			
Key results	18	Summarise key results with reference to study objectives	n/a
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	n/a
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	n/a
Generalisability	21	Discuss the generalisability (external validity) of the study results	n/a
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	10

*Give information separately for exposed and unexposed groups.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

BMJ Open

Screening for familial hypercholesterolaemia in primary school children: protocol for a cross-sectional, feasibility study in Luxemburg city (EARLIE)

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2022-066067.R2
Article Type:	Protocol
Date Submitted by the Author:	07-Nov-2022
Complete List of Authors:	Becker, Marianne; Centre Hospitalier de Luxembourg, Pediatric endocrinology and diabetology (DECCP); VUB University, Research Group GRON Adamski, Aurélie; Centre Hospitalier de Luxembourg, Pediatric endocrinology and diabetology (DECCP) Fandel, Françoise; City of Luxembourg, Department of School Medicine Vaillant, Michel; Luxembourg Institute of Health, Competence Centre for Methodology and Statistics Wagner, Kerstin; Centre Hospitalier de Luxembourg, Department of paediatric cardiology Droste, Dirk; Centre Hospitalier de Luxembourg, Department of Neurology Ziade, Bechara; Luxembourg Ministry of Health, Direction Hein, Steve; Centre Hospitalier de Luxembourg, Department of sports medicine Mendon, Priyanka; Luxembourg Institute of Health, Competence Center for Methodology and Statistics Bocquet, Valéry; Luxembourg Institute of Health, Competence Center for Methodology and Statistics de Beaufort, Carine; Centre Hospitalier de Luxembourg, Department of paediatric endocrinology and diabetology (DECCP); University of Luxembourg - Belval Campus, Faculty of Science, Technology and Medicine
Primary Subject Heading:	Paediatrics
Secondary Subject Heading:	Cardiovascular medicine, Diagnostics, Epidemiology, Health economics
Keywords:	Lipid disorders < DIABETES & ENDOCRINOLOGY, Paediatric endocrinology < DIABETES & ENDOCRINOLOGY, Community child health < PAEDIATRICS, PUBLIC HEALTH, VASCULAR MEDICINE

Screening for familial hypercholesterolaemia in primary school children: protocol for a cross-sectional, feasibility study in Luxemburg city (EARLIE)

Marianne Becker[1,9], Aurélie Adamski[1], Françoise Fandel[2], Michel Vaillant[3], Kerstin Wagner[4], Dirk W. Droste[5], Bechara Ziade[6], Steve Hein[7], Priyanka Mendon[3], Valéry Bocquet[3], Carine de Beaufort[1,8]

[1] Department of paediatric endocrinology and diabetology (DECCP), Centre hospitalier de Luxembourg, Luxembourg
[2] Department of School Medicine, City of Luxembourg, Luxembourg
[3] Competence Center for Methodology and Statistics, Luxembourg Institute of Health, Luxembourg
[4] Department of paediatric cardiology, Centre hospitalier de Luxembourg, Luxembourg
[5] Department of Neurology, Centre Hospitalier de Luxembourg, Luxembourg
[6] Direction of the ministry of Health, Luxembourg
[7] Department of sports medicine, Centre hospitalier de Luxembourg, Luxembourg
[8] Faculty of Science, Technology and Medicine, University of Luxemburg, Esch-Belval, Luxembourg
[9] Vrije Universiteit Brussel (VUB), Research Group GRON, Brussels, Belgium

Corresponding author: becker.marianne@chl.lu

Number of references: 23
Abstract count: 274 words
Word count: 3007 words

Abstract

Introduction Familial hypercholesterolaemia (FH) is a frequent (1:300) autosomal dominantly inherited condition which causes premature (female < 60 years, male < 55 years) cardio-cerebrovascular disease (CVD). Early detection and initiation of treatment can prevent the development of CVD and premature death. Our pilot study aims to investigate the prevalence of FH, the feasibility and efficacy of a screening based on a capillary blood test performed during a school medicine visit in primary school children.

Methods and analysis In this cross-sectional study all children (n=3200) between 7-12 years, attending primary school in the city of Luxemburg, and invited for their mandatory medical school exam between 2021 and 2023, are invited to participate. A study nurse performs a capillary blood test to analyse the lipid profile. Families receive the result including an interpretation and invitation to seek medical advice if indicated. If FH is confirmed, a reverse cascade screening in that family will be proposed. The child will receive standard care. Primary outcome is the occurrence of confirmed FH in the study population. Secondary outcomes include the percentage of children screened, percentage of children with abnormal lipid values, percentage of families screened and percentage of families with additionally identified members suffering from hypercholesterolaemia. A health economic analysis will be performed.

Ethics and dissemination: Ethics approval (reference number 202108/01) has been obtained from the National Research Ethics Committee (CNER (Luxemburg)) and was authorized by the ministry of health in Luxemburg. Families receive written information with an informed consent form. Participation requires an informed consent form signed by the parents. The results will be disseminated in peer-reviewed publications, conference presentations and by public media to the general public.

Study registration number: NCT05271305; clinicaltrials.gov.

Strengths and limitations of this study

- **All school aged children in the city of Luxemburg can be tested with minimal invasive sampling (capillary blood test).**
- **The young age at screening allows prevention of cardiovascular disease in those who test positive for FH and detection and treatment of affected family members via reverse cascade screening before cardio-cerebrovascular events.**
- **Health economic analysis will provide insight in the cost/benefit of a nation-wide screening.**
- **Opt-in approach and recruitment may limit the participation rate and referral to specialist care after detection might not be realised due to underestimation of FH by some paediatricians and family doctors**
- **Confirmation by fasting venous blood samples might decrease the participation rate.**

Introduction

FH is an autosomal dominantly inherited genetic disorder, which causes premature arteriosclerosis leading to cardio- and cerebrovascular disease[1,2]. FH is frequent with an estimated prevalence of 1:276[3] to 1:310[4,5]. Mutations are often found in the low-density lipoprotein receptor gene (*LDLR*), apolipoprotein B gene (*APOB*), or the proprotein convertase subtilisin/kexin type 9 gene (*PCSK9*)[6].

Most of the affected persons are not aware of their condition. A recent publication estimated, that only 10% of affected patients are diagnosed and treated[7]. Vascular pathology develops silently and often FH is not recognised before the first –potentially fatal- heart attack or stroke at a young age (before 40 years). Patients suffering from FH have a standardized incidence ratio to develop a coronary heart disease between the age of 25 and 40 years of 11.1 for men and 17.3 for women[8].

As this development can be avoided by an early diagnosis and treatment with cholesterol lowering medication started in childhood[9,10], FH is a suitable candidate for screening[11]. The cholesterol lowering therapy is available for children from 6 years onwards[12]. A study in England showed that FH screening based on capillary blood tests in 1-2 year old toddlers is cost-effective[13]. At the Technical Meeting of 2021 Slovenian EU presidency, broad professional consensus on paediatric FH screening were presented and public policy recommendations were developed[14]. Despite these facts, so far Slovenia is the only country with a national universal screening program[15].

An opt-out approach – as applied in the Slovenian screening program- would very likely result in a higher participation rate[16], but due to ethical restrictions, only an opt-in approach was feasible for this study.

In Luxemburg – as in many other developed countries- cardiovascular diseases are the leading cause of death[17,18]. Preventing cardiovascular disease will hence not only improve and save lives of affected individuals, but will as well lower the financial burden for the national health care systems: The cost of an universal screening per diagnosed case has been estimated at 2.500 € by Wald et al.[13]. The Luxemburg

Institute of health (LIH) estimated in 2016 the cost of a myocardial infarction survivor in Luxemburg at 15,200 € in the first year and at 2,900 € for every following year. The cost for a cerebrovascular event survivor in Luxemburg was estimated at 19,500 € in the first year and 7,200 € for every following year.

We hypothesize that a screening based on capillary blood tests in the setting of the medical school visit in primary school children will be able to detect affected children and by applying a reverse cascade screening we expect to identify affected family members. We will assess the acceptance of this screening and provide further insight in the cost-effectiveness of this screening approach.

Methods and Analysis

Overview

Cross sectional design, targeting all primary school children (grade 2-6) in the city of Luxemburg. The study will be performed during the mandatory medical school exam (see figure 1). If indicated, further medical follow-up is offered in the National Paediatric Clinic (Diabetes & Endocrine Care Clinic for Pediatric patients, DECCP). The creation of data collection tools and storage as well as the statistical analysis will be delivered by LIH Competence Centre for Methodology and Statistics.

STROBE reporting guidelines were applied in the preparation of this article [19].

Inclusion criteria

- Children, aged 7-12 years, attending primary school classes of the 2nd to 6th grade in Luxemburg City in 2021/2022 and 2022/2023 and who are invited for the medical school exam.
- Written informed consent of the parents/caregivers

Exclusion criteria

- No or an incomplete written informed consent at the medical school visit.

Recruitment

In primary schools, medical school examinations take place every 2 years. The families of children who are invited to the medical school exam, receive written information about the screening and an informed consent form. As the study is running over a period of 2 years, every child will have been invited by the end of the recruitment period.

This information material includes a flyer in 4 languages (see figure 2), a flyer for children adapted to their age (flyer for 7-8 years and flyer 9-12 years), and detailed information about the study for the parents/caregivers.

They will receive as well a questionnaire on the family history of premature cardio-cerebrovascular events and known FH disease (see figure 3).

We will promote this study in order to achieve as high a participation rate as possible and in order to raise awareness for FH. Promotion will include interviews in the lay press, distribution of information material to paediatricians and general practitioners, teachers and the parents' representative committee. National scientific societies of cardiology, neurology, paediatrics and general medicine support the study.

Patient and Public Involvement

The study is in line with the demand of FH patients' groups (FH Europe), to implement a paediatric screening for FH in Europe (https://fheurope.org/policy/prague-declaration/). In the design of the protocol there was no patient involvement, but there is patient involvement and support in the promotion of the study. Dissemination of the study results is planned in scientific journals but equally in lay media in order to enhance awareness of FH.

Study Procedures

A dedicated study nurse will collect the signed informed consent forms and will perform a finger prick using a Medlance® plus special blade (0,8 mm) lancet when the children have their medical school exam (see figure 1).

The nurse will fill out the case record form (CRF, see supplemental material 1), documenting height, weight and blood pressure measurement, data on family history regarding hypercholesterolemia and precocious cardiovascular disease. All data (pseudonymised) will be entered in the online database (developed by the LIH by using

the Vanderbilt REDcap system) for further analysis. Data will be expressed in age adjusted scores.

Lipid profile measurement

The capillary blood sample (15 µl) will be analysed by the Alere Afinion 2 Analyser, using the Alere Afinion™ Lipid Panel. Test result is available in 7 minutes and can be printed. The Alere Afinion™ Lipid Panel includes the analysis of total cholesterol (TC), high-density lipoprotein (HDL) and triglycerides by a colorimetric ELISA method. Based on these results Alere Afinion 2 Analyser will calculate low-density lipoprotein (LDL). A comparison between this handhold machine and CHL (Centre hospitalier de Luxembourg) laboratory method (Colorimetric, enzymatic assays, Roche Cobas 8000) had been conducted and demonstrated a good correlation between the TC, LDL, HDL and triglyceride measurements.

Information for the families

A letter with the result of the lipid test will be sent to the family. This will include the confirmation that the child has a normal result (TC < 200 mg/dl and LDL < 130 mg/dl), or a recommendation to contact their doctor when the cholesterol level is slightly elevated (TC 200-230 mg/dl and/or LDL 130-160 mg/dl). If cholesterol levels are high (TC > 230 mg/dl and/or LDL > 160 mg/dl), the family will receive a letter with an invitation for a further clinical evaluation and the advice to contact their doctor or to make an appointment directly in the paediatric clinic.

As this first blood test is performed non-fasting, applying a calculation of the LDL levels according to Friedewald, high triglyceride levels could lead to falsely elevated LDL levels.

Follow-up in case of high cholesterol levels

When a high cholesterol level is detected, a detailed family history together with a fasting blood test (fast for at least 8 hours) are required.

If the pathological values are confirmed, dietary counselling is indicated followed by a fasting blood control (including TC, LDL, HDL, a genetic analysis for FH panel, lipoprotein(a), serum glutamic oxaloacetic transaminase (GOT), serum glutamic

pyruvic transaminase (GPT), creatine kinase (CK), thyroid-stimulating hormone (TSH)) 3 months later.

Genetic analysis is coordinated and results are interpreted by the genetic department of the Luxemburgish national health laboratory (Laboratoire national de santé, LNS). FH panel is performed at CHU Liège and includes the following genes: *LDLR* (NM_000527.4), *APOB* (NM_000384.2), *PGSK9* (NM_174936.3), *APOE* (NM-a00041.3), *LDLRAP1* (NM_015627.2), *LIPA* (NM_000235.3), *ABCG5* (NM_022436.2), *ABCG8* (NM_022437.2) and *STAP1* (NM_012108.3).

FH is confirmed if[9]:

- genetically confirmed + LDL > 130 mg/dl
- no mutation, but 2x LDL > 190 mg/dl
- no mutation, but 2x LDL >160 mg/dl and precocious cardiovascular diseases in the family

If FH is confirmed,

- cascade screening is offered to the first-degree family members (and if confirmed in those to the related second-degree family members too).
- carotid intima-media thickness (cIMT) measurement before treatment will be performed.
- children will be offered treatment and adult family members will be offered follow-up by adult lipid specialists
- paediatric patients will be offered follow-up once every 6 months with control of the lipid results and adaption of their therapy.

As lipoprotein (a) is an inherited causal risk factor for the development of cardiovascular disease[20], elevated lipoprotein (a) levels (> 50 mg/dl) will guide us to a more aggressive lipid lowering therapy in confirmed FH cases and to initiate a lipid-lowering therapy in borderline cases[21].

cIMT measures will be performed by the same investigator with a Siemens Acuson S2000 device using a 4 to 9 MHz linear probe. Diastolic far-wall common carotid intima-media thickness will be assessed proximally to the bifurcation where the

vessel wall is parallel, using the cursors of the software and following the leading edge system[22].

If indicated, children will be treated with statins, evt. if older than 10 years and insufficient decrease of LDL under statin therapy is achieved, ezetimibe might be added.

If discordant fasting cholesterol levels are obtained (in the two fasting tests) in combination with a negative family history and no genetic mutation, the patient will be offered further follow up including a repeat blood test after several months.

In case of a proven genetic mutation, genetic analysis will be offered to other affected family members.

Data management

A specific electronic CRF (eCRF) is developed for the study with items related to the socio-demographic characteristics of the participants and FH. CRF data will be entered online in the eCRF and the data will be stored in a secured data base.

The database has been designed and the eCRF developed by the Competence Center for Methodology and Statistics of LIH with a GDPR compliant data management system. LIH will be responsible for the data quality control, cleaning and data analysis.

Real time Z-scores for height, BMI and blood pressure for each participant are made available on the e-CRF

For Z-score calculation of height and BMI, L, M and S values are taken from the WHO 2007 growth reference data for children and adolescents (*https://www.who.int/tools/growth-reference-data-for-5to19-years/indicators*).

For Z-scores of systolic and diastolic blood pressure, NHANES reference for the height L, M and S values are used.

All personal and clinical data will be pseudonymised with an ID-number (e.g. IF0001) accessible only to the dedicated employees. The delegation list of all clinical team members will be kept at the DECCP and must be signed by the principal investigator.

All access and changes in the data is tracked and monitored in an audit trail. Therefore, data collection, storage and in-depth analysis respect the highest standards of data protection and security.

Only pseudonymised data without any link to personal data will be accessible by the dedicated members of LIH and collaborating members and partners for analysis and further investigation.

Statistical methods

Sample size calculation

The primary outcome is the prevalence of FH in children between 7-12 years in Luxemburg. The sample size was calculated according to Machin and Campbell[23] based on several indicators i.e. the confidence level, 1 or 2 sided interval, the expected proportion, the aimed precision of the estimated proportion and the population size (from which a simple random sample will be taken without replacement). As a consequence, a two-sided 95% confidence interval for a single proportion using the large sample normal approximation adjusted for a finite population of size of 39,000 (children of 7-12 years in Luxemburg) and a precision of 0.0033 for an expected proportion of 0.005 of FH lead to a sample size equal to 1501.

Sampling plan

The sampling plan will be stratified and randomized with an allocation probability (chance to be selected is equal for all individuals of the same age category and gender) proportional to size (of the population) without replacement (the same individual could not be selected twice). The probability to be sampled nhZhi (nh is the sample size for stratum h, and Zhi is the relative size of unit i in stratum h) will be calculated for each individual based on the size of each age and gender strata in the sample. It will be included as a weighting parameter in the calculation of the prevalence Σ(xi_nhZhi), which will be estimated by summing up data on all patients. These weighting estimations are elements to correct prevalence estimations in case of extreme strata (undersized or oversized stratum).

Statistical analyses

A statistical analysis plan detailing the statistical analysis will be written blinded to the data and before the end of the enrolment.

A check for missing data will be performed. Several methods for processing missing data are possible depending on the type of missing responses (missing completely at random, missing at random or not missing at random).

The primary outcome, prevalence of FH in children between 7-12 years in Luxemburg, will be calculated by summing up data on all patients and weighting by the size of each age and gender strata as described earlier.

To include a finite population correction in Taylor series variance estimation, the size of each age and gender strata in the target population will also be entered in the analyses in order to calculate the Wald 95% confidence interval, and thereby extrapolate the proportion of FH to the target population. The surveyfreq procedure of the statistical software SAS System V9.3 (SAS Institute, Cary, NC) will be used.

To complete corrections on prevalence provided by the weighting process, adjustments on age and sex are planned in order to estimate the prevalence. These adjustments will also take into account discrepancies between theoretical and real numbers for each stratum as well as non-responses issue.

Secondary outcomes be calculated in a similar way as the primary outcome, if possible, in order to enable inference to the target population of children between 7-12 years in Luxemburg.

The secondary outcomes are:

- Percentage of children screened (number screened/number invited)
- Percentage of children with abnormal values
- Percentage of children with confirmed hypercholesterolaemia and treatment
- Percentage of families screened
- Percentage of families with additional family members with confirmed hypercholesterolaemia and treatment

Ethics and Dissemination

This study has received approval from the National Research Ethics Committee in Luxemburg (202108/01) and the Ministry of Health.

All parents/guardians will be provided with detailed written information about the study procedure. Written informed consent is necessary in order to include a child in this study. All results will be published anonymously to ensure that no participant can be identified. The name and identity of the participants will not appear in any of the published materials.

The results of this study will be disseminated in medical and scientific pre-reviewed publications and conference presentations.

Footnotes

- **Contributors:** MB and CdB conceived the study. MB, CdB, KW, DWD, SH, FF, BZ initiated the study design and AA helped with the implementation. MB and CdB are grant holders. MV, PM and VB provided statistical expertise in clinical study design. All authors contributed to refinement of the study protocol and approved the final manuscript.
- **Acknowledgement:** We thank Michael Witsch who has helped to set up the z-score calculation for height, bmi and blood pressure.
- **Funding statement:** This work was supported by the non-profit organization “Coeur- Daniel Wagner”, grant number: N/A.
- **Competing interests statement.**
  MB and DWD participated in a scientific advisory board of Daiichi Sankyo.
  AA, SH FF, MV, KW, BZ, SH, PM, VC and CdB have nothing to declare.

References

1 Austin MA, Hutter CM, Zimmern RL, *et al.* Familial hypercholesterolemia and coronary heart disease: a HuGE association review. *Am J Epidemiol* 2004;**160**:421–9. doi:10.1093/aje/kwh237

2 Ference BA, Ginsberg HN, Graham I, *et al.* Low-density lipoproteins cause atherosclerotic cardiovascular disease. 1. Evidence from genetic, epidemiologic,

and clinical studies. A consensus statement from the European Atherosclerosis Society Consensus Panel. *European Heart Journal* 2017;**38**:2459–72. doi:10.1093/eurheartj/ehx144

3 Pang J, Martin AC, Mori TA, *et al.* Prevalence of Familial Hypercholesterolemia in Adolescents: Potential Value of Universal Screening? *J Pediatr* 2016;**170**:315–6. doi:10.1016/j.jpeds.2015.11.019

4 Hu P, Dharmayat KI, Stevens CAT, *et al.* Prevalence of Familial Hypercholesterolemia Among the General Population and Patients With Atherosclerotic Cardiovascular Disease. *Circulation* 2020;**141**:1742–59. doi:10.1161/CIRCULATIONAHA.119.044795

5 Beheshti SO, Madsen CM, Varbo A, *et al.* Worldwide Prevalence of Familial Hypercholesterolemia: Meta-Analyses of 11 Million Subjects. *Journal of the American College of Cardiology* 2020;**75**:2553–66. doi:10.1016/j.jacc.2020.03.057

6 Austin MA, Hutter CM, Zimmern RL, *et al.* Genetic causes of monogenic heterozygous familial hypercholesterolemia: a HuGE prevalence review. *Am J Epidemiol* 2004;**160**:407–20. doi:10.1093/aje/kwh236

7 Representatives of the Global Familial Hypercholesterolemia Community. Reducing the Clinical and Public Health Burden of Familial Hypercholesterolemia: A Global Call to Action. *JAMA Cardiology* 2020;**5**:217–29. doi:10.1001/jamacardio.2019.5173

8 Mundal LJ, Igland J, Veierød MB, *et al.* Impact of age on excess risk of coronary heart disease in patients with familial hypercholesterolaemia. *Heart* 2018;**104**:1600–7. doi:10.1136/heartjnl-2017-312706

9 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. doi:10.1093/eurheartj/ehv157

10 Luirink IK, Wiegman A, Kusters DM, *et al.* 20-Year Follow-up of Statins in Children with Familial Hypercholesterolemia. *N Engl J Med* 2019;**381**:1547–56. doi:10.1056/NEJMoa1816454

11 Kusters DM, de Beaufort C, Widhalm K, *et al.* Paediatric screening for hypercholesterolaemia in Europe. *Arch Dis Child* 2012;**97**:272–6. doi:10.1136/archdischild-2011-300081

12 Vuorio A, Kuoppala J, Kovanen PT, *et al.* Statins for children with familial hypercholesterolemia. *Cochrane Database Syst Rev* 2017;**7**:CD006401. doi:10.1002/14651858.CD006401.pub4

13 Wald DS, Bestwick JP, Morris JK, *et al.* Child-Parent Familial Hypercholesterolemia Screening in Primary Care. *N Engl J Med* 2016;**375**:1628–37. doi:10.1056/NEJMoa1602777

14 Groselj U, Wiegman A, Gidding SS. Screening in children for familial hypercholesterolaemia: start now. *European Heart Journal* 2022;:ehac224. doi:10.1093/eurheartj/ehac224

15 Groselj 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. doi:10.1016/j.atherosclerosis.2018.06.858

16 Sustar U, Kordonouri O, Mlinaric M, *et al.* Universal screening for familial hypercholesterolemia in 2 populations. *Genet Med* 2022;:S1098-3600(22)00819-X. doi:10.1016/j.gim.2022.06.010

17 Scharel L. Statistiques des causes de décès pour l'année 2019. ;:84.

18 Global Health Estimates: Life expectancy and leading causes of death and disability. https://www.who.int/data/gho/data/themes/mortality-and-global-health-estimates (accessed 26 Feb 2022).

19 von Elm E, Altman DG, Egger M, *et al.* The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement: guidelines for reporting observational studies. *Lancet* 2007;**370**:1453–7. doi:10.1016/S0140-6736(07)61602-X

20 Welsh P, Welsh C, Celis-Morales CA, *et al.* Lipoprotein(a) and cardiovascular disease: prediction, attributable risk fraction, and estimating benefits from novel interventions. *European Journal of Preventive Cardiology* 2022;**28**:1991–2000. doi:10.1093/eurjpc/zwaa063

21 Catapano AL, Daccord M, Damato E, *et al.* How should public health recommendations address Lp(a) measurement, a causative risk factor for cardiovascular disease (CVD)? *Atherosclerosis* 2022;**349**:136–43. doi:10.1016/j.atherosclerosis.2022.02.013

22 Droste DW, Schulte V, Schomacher G, *et al.* Comparison of Automatic Intima Media Thickness Measurement and Eye Balling. *Cerebrovasc Dis* 2010;**29**:307–8. doi:10.1159/000278697

23 Machin D, etc, Campbell MJ. *Statistical tables for the design of clinical trials*. Illustrated edition. Oxford England ; Boston : St Louis, Mo: : Blackwell Scientific Publications 1987.

Figure legends:

- Figure 1: Study flowchart. TC: total cholesterol; LDL: low-density lipoprotein; precocious CVD: cardiovascular disease < 60 years in males/ < 55 years in females; IMT: intima media thickness; GP: general practitioner

- Figure 2: Information material: Flyer in 4 languages. Reproduced with permission from Centre hospitalier de Luxembourg.
- Figure 3: English part of the questionnaire for parents.

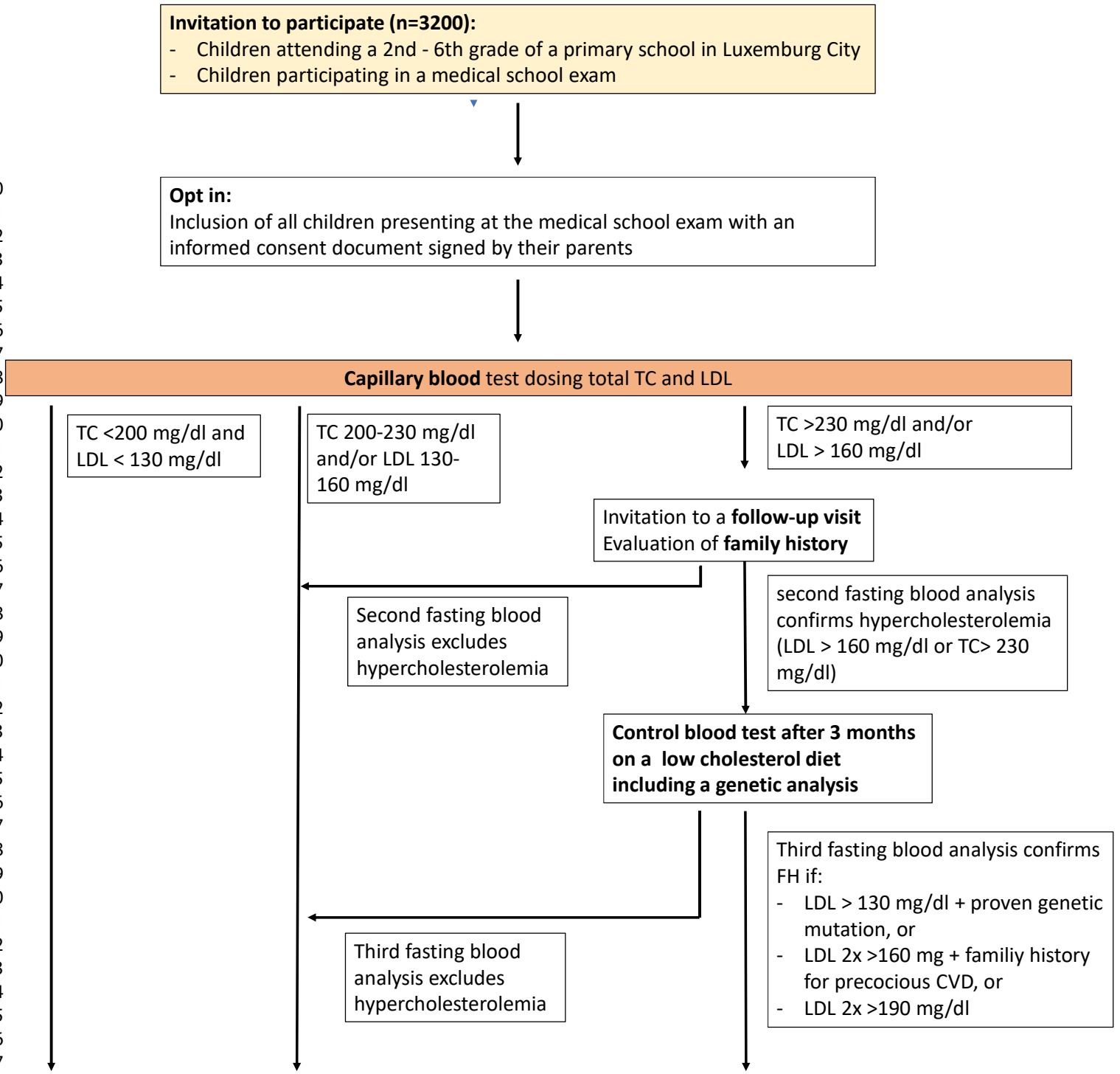

Figure 1 Study flowchart. TC: total cholesterol; LDL: low-density lipoprotein; precocious CVD: cardiovascular disease < 60 years in males/ < 55 years in females; IMT: intima media thickness; GP: general practicioner

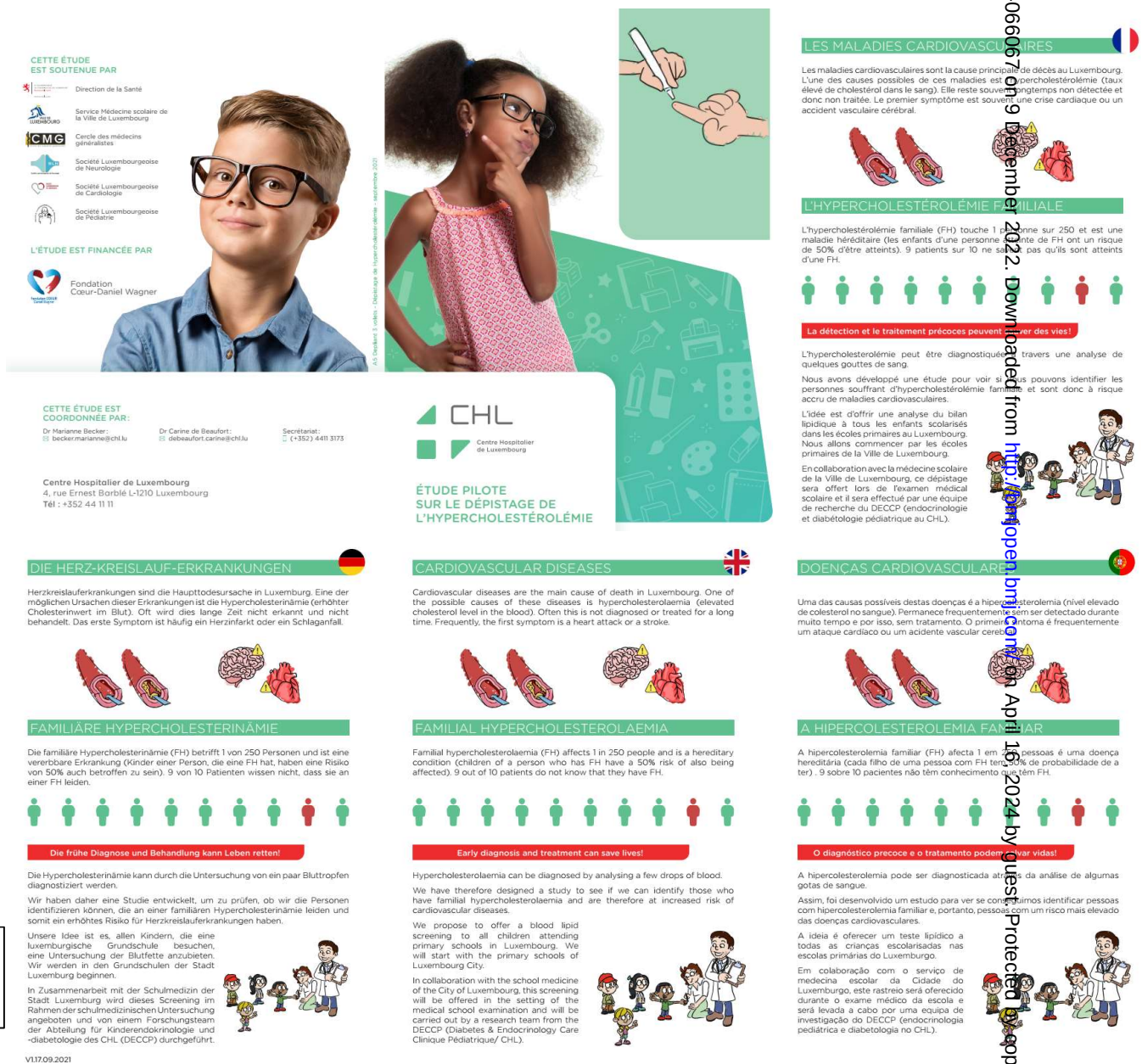

Figure 2
Information material: Flyer in 4 languages

Questionnaire for parents in the setting of the hypercholesterolemia screening study:

- Do you have an elevated cholesterol level?

  Father: ☐ Yes ☐ No ☐ I do not know my cholesterol level.

  If yes: Are you receiving medication for it? ☐ Yes ☐ No

  Mother: ☐ Yes ☐ No ☐ I do not know my cholesterol level.

  If yes: Are you receiving medication for it? ☐ Yes ☐ No

- Does someone in your family suffer or suffered from a cardiovascular disease (heart attack, stroke) already at a young age (women < 60 years, men < 55 years)?

  ☐ Yes ☐ No

  - If yes, who? _____

Figure 3: English part of the questionnaire for parents

Supplemental material 1: CRF

CRF

Case report form,			Patient number
	FH Pilot national depistage		
Date of Birth		month	year
gender		☐male	☐female
weight (kg)			
height (cm)			
blood pressure (mmHg)			
Family Questionnaire		☐ yes	☐ no
if yes: high cholesterol father:	☐ yes	☐ no	☐ unknown
if yes: high cholesterol mother:	☐ yes	☐ no	☐ unknown
if yes: precocious CVD:		☐ yes	☐ no
if precocious CVD: who*:			
blood sample		☐ yes	☐ no
Time of sampling	day	month	hour
LABORATORY OUTCOME			
	result		ref range
Total cholesterol (mg/dl)			< 200
LDL cholesterol (mg/dl)			< 130
HDL Cholesterol (mg/dl)			> 45
triglycerides (mg/dl)			< 130
Further action	☐ normal result -> result transmitted to family		
	☐ slightly elevated -> result transmitted to family + proposition to see GP		
	☐ abnormal result -> result transmitted to family + invitation DECCP/see GP		
* GMM = grand-mère maternelle, GMP = grand-père paternel, TM = tante maternelle; TP = tante paternel			
CM = cousin/e maternel(le), CP = cousin/e paternel(le)			

eCRF:

Out of range alerts are available in the e-CRF for TC and LDL values. The alerts confirm if the values for the participant are normal, slightly abnormal or abnormal. Specific validation checks are created in e-CRF for discrepancies between normal and abnormal laboratory values and the follow up action for the families. For example, if TC and LDL values are abnormal, but the follow-up action is selected as "*Normal Result- Result transmitted to family*", a query would pop up, alerting this discrepancy

STROBE Statement—Checklist of items that should be included in reports of ***cross-sectional studies***

	Item No	Recommendation	Page No
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	1
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	2-3
Objectives	3	State specific objectives, including any prespecified hypotheses	3
Methods			
Study design	4	Present key elements of study design early in the paper	4
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	4-7
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants	4
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	8,9
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	*5-7*
Bias	9	Describe any efforts to address potential sources of bias	8
Study size	10	Explain how the study size was arrived at	7,8
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	6,7
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	8
		(*b*) Describe any methods used to examine subgroups and interactions	n/a
		(*c*) Explain how missing data were addressed	8
		(*d*) If applicable, describe analytical methods taking account of sampling strategy	8
		(*e*) Describe any sensitivity analyses	8
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	n/a
		(b) Give reasons for non-participation at each stage	n/a
		(c) Consider use of a flow diagram	Figure 1
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	n/a
		(b) Indicate number of participants with missing data for each variable of interest	n/a
Outcome data	15*	Report numbers of outcome events or summary measures	n/a

Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	n/a
		(*b*) Report category boundaries when continuous variables were categorized	n/a
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	n/a
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	n/a
Discussion			
Key results	18	Summarise key results with reference to study objectives	n/a
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	n/a
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	n/a
Generalisability	21	Discuss the generalisability (external validity) of the study results	n/a
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	10

*Give information separately for exposed and unexposed groups.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.