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

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

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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.

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3 Families receive written information with an informed consent form. Children, who
4 come to the medical visit with the signed informed consent form, are included. The
5 study nurse performs a capillary blood test. Families receive the result including an
6 interpretation and invitation to seek medical advice if indicated. If familial
7 hypercholesterolaemia is confirmed, a cascade screening in that family will be
8 proposed. The child will receive standard care.
9

10 Primary outcome is the occurrence of confirmed familial hypercholesterolaemia in the
11 study population. Secondary outcomes include the percentage of children screened,
12 percentage of children with abnormal lipid values, percentage of families screened and
13 percentage of families with additionally identified members suffering from
14 hypercholesterolaemia. A health economic analysis will be performed.
15
16

17 **Ethics and dissemination:** Ethics approval has been obtained from the National
18 Research Ethics Committee (CNER (Luxemburg)) and was authorized by the ministry
19 of health in Luxemburg. The results will be disseminated in peer-reviewed publications,
20 conference presentations and by public media to the general public.
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22

23 **Trial registration number:** NCT05271305; clinicaltrials.gov.
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31 **Strengths of this study**

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

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

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3 Familial hypercholesterolaemia (FH) is an autosomal dominantly inherited genetic
4 disorder, which causes premature arteriosclerosis leading to cardio- and
5 cerebrovascular disease. FH is frequent with an estimated prevalence of 1:217[1] to
6 1:250[2]. Mutations are often found in the low-density lipoprotein receptor gene (LDLr),
7 apolipoprotein B gene (APOB), or the proprotein convertase subtilisin/kexin type 9
8 gene (PCSK9).
9

10 Most of the affected persons are not aware of their condition. A recent publication
11 estimated, that only 10% of affected patients are diagnosed and treated [3]. Vascular
12 pathology develops silently and often FH is not recognised before the first –potentially
13 fatal- heart attack or stroke at a young age (before 40 years). Patients suffering from
14 FH have a 100 fold higher risk to die from a coronary heart disease at the age of 20-
15 39 years[4].
16

17 As this development can be avoided by an early diagnosis and treatment with
18 cholesterol lowering medication started in childhood[5,6], FH is a suitable candidate
19 for screening[7]. The cholesterol lowering therapy is available for children from 6 years
20 onwards[8]. A study in England showed that FH screening based on capillary blood
21 tests in 1-2 year old toddlers is cost-effective[9]. At the Technical Meeting of 2021
22 Slovenian EU presidency, broad professional consensus on paediatric FH screening
23 were presented and public policy recommendations were developed[10]. Despite
24 these facts, so far Slovenia is the only country with a national universal screening
25 program[11].
26

27 In Luxemburg – as in many other developed countries- cardiovascular diseases are
28 the leading cause of death[12,13]. Preventing cardiovascular disease will hence not
29 only improve and save lives of affected individuals, but will as well lower the financial
30 burden for the national health care systems: The cost of an universal screening per
31 diagnosed case has been estimated at 2.500 € by Wald et al.[9]. The Luxemburg
32 Institute of health (LIH) estimated in 2016 the cost of a myocardial infarction survivor
33 in Luxemburg at 15,200 € in the first year and at 2,900 € for every following year. The
34 cost for a cerebrovascular event survivor in Luxemburg was estimated at 19,500 € in
35 the first year and 7,200 € for every following year.
36

37 We hypothesize that a screening based on capillary blood tests in the setting of the
38 medical school visit in primary school children will be able to detect affected children
39 and by applying the cascade screening we expect to identify affected family members.
40

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.

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3 They will receive as well a questionnaire on the family history of premature cardio-
4 cerebrovascular events and known FH disease (see figure 3).
5
6

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

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16 Participants were not directly involved in the study design. But patients were involved
17 in the dissemination of the study by giving an interview explaining the interest of our
18 study and patients and public are a main target of the dissemination of our results in
19 order to enhance awareness for FH.
20
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23 **Study Procedures**

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26 A dedicated study nurse will collect the signed informed consent forms and will perform
27 the finger prick when the children have their medical school exam (see figure 1).
28
29

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

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42 The capillary blood sample (15 µl) will be analysed by the Alere Afinion 2 Analyser,
43 using the Alere Afinion Lipid Panel. Test result is available in 7 minutes and can be
44 printed. The Alere Afinion Lipid Panel includes the analysis of total cholesterol, HDL
45 and triglycerides by a colorimetric ELISA method. Based on these results Alere Afinion
46 2 Analyser will calculate LDL. A comparison between this handheld machine and CHL
47 (Centre hospitalier de Luxembourg) laboratory method (Colorimetric, enzymatic
48 assays, Roche Cobas 8000) had been conducted and demonstrated a good correlation
49 between the Cholesterol, LDL, HDL and triglyceride measurements.
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57 ***Information for the families***

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3 A letter with the result of the lipid test will be sent to the family. This will include the
4 confirmation that the child has a normal result (total cholesterol (CT) < 200 mg/dl and
5 LDL cholesterol (LDLC) < 130 mg/dl), or a recommendation to contact their doctor
6 when the cholesterol level is slightly elevated (CT 200-230 mg/dl and/or LDLC 130-
7 160 mg/dl). If cholesterol levels are high (CT > 230 mg/dl and/or LDLC > 160 mg/dl),
8 the family will receive a letter with an invitation for a further clinical evaluation and the
9 advice to contact their doctor or to make an appointment directly in the paediatric clinic.
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15 16 **Follow-up in case of high cholesterol levels**

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18 When a high cholesterol level is detected, a detailed family history together with fasting
19 blood tests are required.
20

21
22 If the pathological values are confirmed, dietary counselling is indicated followed by a
23 fasting blood control (including a genetic analysis for FH panel, lipoprotein(a), GOT,
24 GPT, CK, TSH) 3 months later.
25
26

27
28 Familial hypercholesterolaemia is confirmed if:
29

- 30 • genetically confirmed + LDL cholesterol > 130 mg/dl
- 31 • no mutation, but 2x LDL cholesterol > 190 mg/dl
- 32 • no mutation, but 2x LDL cholesterol > 160 mg/dl and precocious cardiovascular
33 diseases in the family
34
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38
39 If FH is confirmed,
40

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

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56 A specific electronic CRF (eCRF) is developed for the study with items related to the
57 socio-demographic characteristics of the participants and familial
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1
2
3 hypercholesterolaemia. CRF data will be entered online in the eCRF and the data will
4 be stored in a secured data base.
5

6
7 The database has been designed and the eCRF developed by the Competence Center
8 for Methodology and Statistics of LIH with a GDPR compliant data management
9 system. LIH will be responsible for the data quality control, cleaning and data analysis.
10
11

12
13 Real time Z-scores for height, BMI and blood pressure for each participant are made
14 available on the e-CRF
15

16
17 For Z-score calculation of height and BMI, L, M and S values are taken from the WHO
18 2007 growth reference data for children and adolescents
19 (<https://www.who.int/tools/growth-reference-data-for-5to19-years/indicators>).
20
21

22
23 For Z-scores of systolic and diastolic blood pressure, NHANES reference for the height
24 L, M and S values are used.
25

26
27 Out of range alerts are available in the e-CRF for total cholesterol and LDL cholesterol
28 values. The alerts confirm if the values for the participant are normal, slightly abnormal
29 or abnormal. Specific validation checks are created in e-CRF for discrepancies
30 between normal and abnormal laboratory values and the follow up action for the
31 families. For example, if total cholesterol and LDL values are abnormal, but the follow-
32 up action is selected as “*Normal Result- Result transmitted to family*”, a query would
33 pop up, alerting this discrepancy
34
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36
37 All personal and clinical data will be pseudonymised with an ID-number (e.g. IF0001)
38 accessible only to the dedicated employees. The delegation list of all clinical team
39 members will be kept at the DECCP and must be signed by the principal investigator.
40 All access and changes in the data is tracked and monitored in an audit trail. Therefore,
41 data collection, storage and in-depth analysis respect the highest standards of data
42 protection and security.
43
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46 Only pseudonymised data without any link to personal data will be accessible by the
47 dedicated members of LIH and collaborating members and partners for analysis and
48 further investigation.
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51 52 53 54 55 56 57 58 **Statistical methods** 59 60

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 $\sum(x_i_{nhZhi})$, 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.

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3 Secondary outcomes be calculated in a similar way as the primary outcome if possible
4 in order to enable inference to the target population of children between 7-12 years in
5 Luxembourg.
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8
9 The secondary outcomes are:

- 10
11
- 12 • Percentage of children screened (number screened/number invited)
 - 13 • Percentage of children with abnormal values
 - 14 • Percentage of children with confirmed hypercholesterolaemia and treatment
 - 15 • Percentage of families screened
 - 16 • Percentage of families with additional family members with confirmed
 - 17 hypercholesterolaemia and treatment
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26 **Ethics and Dissemination**

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

30
31
32 All parents/guardians will be provided with detailed written information about the study
33 procedure. Written informed consent is necessary in order to include a child in this
34 study. All results will be published anonymously to ensure that no participant can be
35 identified. The name and identity of the participants will not appear in any of the
36 published materials.
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41 The results of this study will be disseminated in medical and scientific pre-reviewed
42 publications and conference presentations.
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48 **Footnotes**

- 49
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- 51 • **Contributors:** MB and CdB conceived the study. MB, CdB, KW, DWD, SH,
52 FF, BZ initiated the study design and AA helped with the implementation. MB
53 and CdB are grant holders. MV, PM and VB provided statistical expertise in
54 clinical trial design. All authors contributed to refinement of the study protocol
55 and approved the final manuscript.
56
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- 2
- 3 • **Acknowledgement:** We thank Michael Witsch who has helped to set up the
- 4 z-score calculation for height, bmi and blood pressure.
- 5
- 6 • **Funding statement:** This work was supported by the non-profit organization
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- 8
- 9 • **Competing interests statement.**
- 10 MB and DWD participated in a scientific advisory board of Daiichi Sankyo.
- 11
- 12 AA, SH FF, MV, KW, BZ, SH, PM, VC and CdB have nothing to declare.
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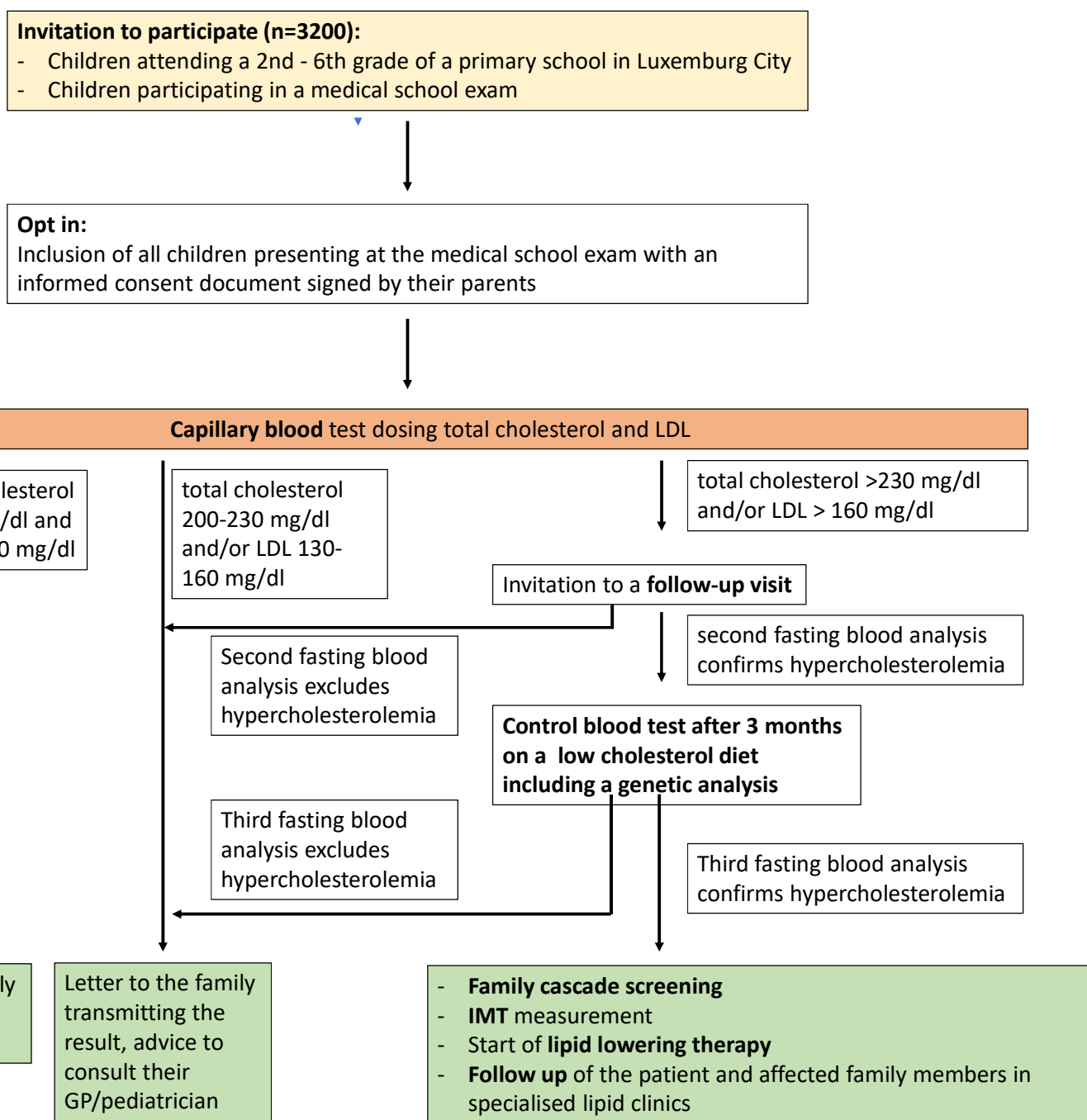


Figure 1 Study flowchart. IMT, intima media thickness

CETTE ÉTUDE EST SOUTENUE PAR

- Direction de la Santé
- Service Médecine scolaire de la Ville de Luxembourg
- CMG Cercle des médecins généralistes
- Société Luxembourgeoise de Neurologie
- Société Luxembourgeoise de Cardiologie
- Société Luxembourgeoise de Pédiatrie

L'ÉTUDE EST FINANÇÉE PAR

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ÉTUDE PILOTE SUR LE DÉPISTAGE DE L'HYPERCHOLESTÉROLÉMIE

DIE HERZ-KREISLAUF-ERKRANKUNGEN

Herz-Kreislauferkrankungen sind die Haupttodesursache in Luxemburg. Eine der möglichen Ursachen dieser Erkrankungen ist die Hypercholesterinämie (erhöhter Cholesterinwert im Blut). Oft wird dies lange Zeit nicht erkannt und nicht behandelt. Das erste Symptom ist häufig ein Herzinfarkt oder ein Schlaganfall.

CARDIOVASCULAR DISEASES

Cardiovascular diseases are the main cause of death in Luxembourg. One of the possible causes of these diseases is hypercholesterolaemia (elevated cholesterol level in the blood). Often this is not diagnosed or treated for a long time. Frequently, the first symptom is a heart attack or a stroke.

DOENÇAS CARDIOVASCULARES

Uma das causas possíveis destas doenças é a hipercolesterolemia (nível elevado de colesterol no sangue). Permanece frequentemente sem ser detectado durante muito tempo e por isso, sem tratamento. O primeiro sintoma é frequentemente um ataque cardíaco ou um acidente vascular cerebral.

FAMILIÄRE HYPERCHOLESTERINÄMIE

Die familiäre Hypercholesterinämie (FH) betrifft 1 von 250 Personen und ist eine vererbare Erkrankung (Kinder einer Person, die eine FH hat, haben ein Risiko von 50% auch betroffen zu sein). 9 von 10 Patienten wissen nicht, dass sie an einer FH leiden.

FAMILIAL HYPERCHOLESTEROLAEMIA

Familial hypercholesterolaemia (FH) affects 1 in 250 people and is a hereditary condition (children of a person who has FH have a 50% risk of also being affected). 9 out of 10 patients do not know that they have FH.

A HIPERCOLESTEROLEMIA FAMILIAR

A hipercolesterolemia familiar (FH) afecta 1 em 250 pessoas e é uma doença hereditária (cada filho de uma pessoa com FH tem 50% de probabilidade de a ter). 9 sobre 10 pacientes não têm conhecimento de que têm FH.

Die frühe Diagnose und Behandlung kann Leben retten!

Die Hypercholesterinämie kann durch die Untersuchung von ein paar Blutropfen diagnostiziert werden.

Wir haben daher eine Studie entwickelt, um zu prüfen, ob wir die Personen identifizieren können, die an einer familiären Hypercholesterinämie leiden und somit ein erhöhtes Risiko für Herz-Kreislauferkrankungen haben.

Unsere Idee ist es, allen Kindern, die eine luxemburgische Grundschule besuchen, eine Untersuchung der Blutfette anzubieten. Wir werden in den Grundschulen der Stadt Luxemburg beginnen.

In Zusammenarbeit mit der Schulmedizin der Stadt Luxemburg wird dieses Screening im Rahmen der schulmedizinischen Untersuchung angeboten und von einem Forschungsteam der Abteilung für Kinderendokrinologie und -diabetologie des CHL (DECCP) durchgeführt.

Early diagnosis and treatment can save lives!

Hypercholesterolaemia can be diagnosed by analysing a few drops of blood.

We have therefore designed a study to see if we can identify those who have familial hypercholesterolaemia and are therefore at increased risk of cardiovascular diseases.

We propose to offer a blood lipid screening to all children attending primary schools in Luxembourg. We will start with the primary schools of Luxembourg City.

In collaboration with the school medicine of the City of Luxembourg, this screening will be offered in the setting of the medical school examination and will be carried out by a research team from the DECCP (Diabetes & Endocrinology Care Clinique Pédiatrique/ CHL).

O diagnóstico precoce e o tratamento podem salvar vidas!

A hipercolesterolemia pode ser diagnosticada através da análise de algumas gotas de sangue.

Assim, foi desenvolvido um estudo para ver se conseguimos identificar pessoas com hipercolesterolemia familiar e, portanto, pessoas com um risco mais elevado das doenças cardiovasculares.

A ideia é oferecer um teste lipídico a todas as crianças escolarizadas nas escolas primárias do Luxemburgo.

Em colaboração com o serviço de medicina escolar da Cidade de Luxemburgo, este rastreio será oferecido durante o exame médico da escola e será levada a cabo por uma equipa de investigação do DECCP (endocrinologia pediátrica e diabetologia no CHL).

Figure 2 Information material: Flyer in 3 languages

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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

1			
2	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
3			n/a
4			
5			
6			(b) Report category boundaries when continuous variables were categorized
7			n/a
8			
9			(c) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period
10			n/a
11	Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses
12			n/a
13			
14	Discussion		
15	Key results	18	Summarise key results with reference to study objectives
16			n/a
17	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
18			n/a
19			
20	Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence
21			n/a
22			
23			
24	Generalisability	21	Discuss the generalisability (external validity) of the study results
25			n/a
26	Other information		
27	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
28			10
29			
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*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)

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

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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

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

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

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26 **Study registration number:** NCT05271305; clinicaltrials.gov.

27 28 29 30 31 **Strengths and limitations of this study**

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

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3 cost for a cerebrovascular event survivor in Luxemburg was estimated at 19,500 € in
4 the first year and 7,200 € for every following year.
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7 We hypothesize that a screening based on capillary blood tests in the setting of the
8 medical school visit in primary school children will be able to detect affected children
9 and by applying a reverse cascade screening we expect to identify affected family
10 members. We will assess the acceptance of this screening and provide further insight
11 in the cost-effectiveness of this screening approach.
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19 **Methods and Analysis**

22 **Overview**

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25 Cross sectional design, targeting all primary school children (grade 2-6) in the city of
26 Luxemburg. The study will be performed during the mandatory medical school exam
27 (see figure 1). If indicated, further medical follow-up is offered in the National Paediatric
28 Clinic (Diabetes & Endocrine Care Clinic for Pediatric patients, DECCP). The creation
29 of data collection tools and storage as well as the statistical analysis will be delivered
30 by LIH Competence Centre for Methodology and Statistics.
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36 STROBE reporting guidelines were applied in the preparation of this article [19].
37
38

39 **Inclusion criteria**

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41
42 - Children, aged 7-12 years, attending primary school classes of the 2nd to 6th
43 grade in Luxemburg City in 2021/2022 and 2022/2023 and who are invited for
44 the medical school exam.
45
46 - Written informed consent of the parents/caregivers
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48

49 **Exclusion criteria**

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52 - No or an incomplete written informed consent at the medical school visit.
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54

55 **Recruitment**

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57 In primary schools, medical school examinations take place every 2 years. The families
58 of children who are invited to the medical school exam, receive written information
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3 about the screening and an informed consent form. As the study is running over a
4 period of 2 years, every child will have been invited by the end of the recruitment
5 period.
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9 This information material includes a flyer in 4 languages (see figure 2), a flyer for
10 children adapted to their age (flyer for 7-8 years and flyer 9-12 years), and detailed
11 information about the study for the parents/caregivers.
12
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15 They will receive as well a questionnaire on the family history of premature cardio-
16 cerebrovascular events and known FH disease (see figure 3).
17
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19 We will promote this study in order to achieve as high a participation rate as possible
20 and in order to raise awareness for FH. Promotion will include interviews in the lay
21 press, distribution of information material to paediatricians and general practitioners,
22 teachers and the parents' representative committee. National scientific societies of
23 cardiology, neurology, paediatrics and general medicine support the study.
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29 **Patient and Public Involvement**

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

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45 A dedicated study nurse will collect the signed informed consent forms and will perform
46 a finger prick using a Medlance® plus special blade (0,8 mm) lancet when the children
47 have their medical school exam (see figure 1).
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51 The nurse will fill out the case record form (CRF, see supplemental material 1),
52 documenting height, weight and blood pressure measurement, data on family history
53 regarding hypercholesterolemia and precocious cardiovascular disease. All data
54 (pseudonymised) will be entered in the online database (developed by the LIH by using
55 the Vanderbilt REDcap system) for further analysis. Data will be expressed in age
56 adjusted scores.
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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 handheld 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].

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3 If indicated, children will be treated with statins, evt. if older than 10 years and
4 insufficient decrease of LDL under statin therapy is achieved, ezetimibe might be
5 added.
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9 If discordant fasting cholesterol levels are obtained (in the two fasting tests) in
10 combination with a negative family history and no genetic mutation, the patient will be
11 offered further follow up including a repeat blood test after several months.
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15 In case of a proven genetic mutation, genetic analysis will be offered to other affected
16 family members.
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19 20 21 22 **Data management**

23
24 A specific electronic CRF (eCRF) is developed for the study with items related to the
25 socio-demographic characteristics of the participants and FH. CRF data will be entered
26 online in the eCRF and the data will be stored in a secured data base.
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30 The database has been designed and the eCRF developed by the Competence Center
31 for Methodology and Statistics of LIH with a GDPR compliant data management
32 system. LIH will be responsible for the data quality control, cleaning and data analysis.
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36 Real time Z-scores for height, BMI and blood pressure for each participant are made
37 available on the e-CRF
38
39

40
41 For Z-score calculation of height and BMI, L, M and S values are taken from the WHO
42 2007 growth reference data for children and adolescents
43 (<https://www.who.int/tools/growth-reference-data-for-5to19-years/indicators>).
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47 For Z-scores of systolic and diastolic blood pressure, NHANES reference for the height
48 L, M and S values are used.
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51 All personal and clinical data will be pseudonymised with an ID-number (e.g. IF0001)
52 accessible only to the dedicated employees. The delegation list of all clinical team
53 members will be kept at the DECCP and must be signed by the principal investigator.
54 All access and changes in the data is tracked and monitored in an audit trail. Therefore,
55 data collection, storage and in-depth analysis respect the highest standards of data
56 protection and security.
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3 Only pseudonymised data without any link to personal data will be accessible by the
4 dedicated members of LIH and collaborating members and partners for analysis and
5 further investigation.
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10 11 **Statistical methods**

12 13 ***Sample size calculation***

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16 The primary outcome is the prevalence of FH in children between 7-12 years in
17 Luxemburg. The sample size was calculated according to Machin and Campbell[23]
18 based on several indicators i.e. the confidence level, 1 or 2 sided interval, the expected
19 proportion, the aimed precision of the estimated proportion and the population size
20 (from which a simple random sample will be taken without replacement). As a
21 consequence, a two-sided 95% confidence interval for a single proportion using the
22 large sample normal approximation adjusted for a finite population of size of 39,000
23 (children of 7-12 years in Luxemburg) and a precision of 0.0033 for an expected
24 proportion of 0.005 of FH lead to a sample size equal to 1501.
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33 34 ***Sampling plan***

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36 The sampling plan will be stratified and randomized with an allocation probability
37 (chance to be selected is equal for all individuals of the same age category and gender)
38 proportional to size (of the population) without replacement (the same individual could
39 not be selected twice). The probability to be sampled $n_h Z_{hi}$ (n_h is the sample size for
40 stratum h , and Z_{hi} is the relative size of unit i in stratum h) will be calculated for each
41 individual based on the size of each age and gender strata in the sample. It will be
42 included as a weighting parameter in the calculation of the prevalence $\sum(x_i n_h Z_{hi})$,
43 which will be estimated by summing up data on all patients. These weighting
44 estimations are elements to correct prevalence estimations in case of extreme strata
45 (undersized or oversized stratum).
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54 55 ***Statistical analyses***

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57 A statistical analysis plan detailing the statistical analysis will be written blinded to the
58 data and before the end of the enrolment.
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3 A check for missing data will be performed. Several methods for processing missing
4 data are possible depending on the type of missing responses (missing completely at
5 random, missing at random or not missing at random).
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9 The primary outcome, prevalence of FH in children between 7-12 years in Luxemburg,
10 will be calculated by summing up data on all patients and weighting by the size of each
11 age and gender strata as described earlier.
12
13

14
15 To include a finite population correction in Taylor series variance estimation, the size
16 of each age and gender strata in the target population will also be entered in the
17 analyses in order to calculate the Wald 95% confidence interval, and thereby
18 extrapolate the proportion of FH to the target population. The surveyfreq procedure of
19 the statistical software SAS System V9.3 (SAS Institute, Cary, NC) will be used.
20
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22

23
24 To complete corrections on prevalence provided by the weighting process,
25 adjustments on age and sex are planned in order to estimate the prevalence. These
26 adjustments will also take into account discrepancies between theoretical and real
27 numbers for each stratum as well as non-responses issue.
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32 Secondary outcomes be calculated in a similar way as the primary outcome, if possible,
33 in order to enable inference to the target population of children between 7-12 years in
34 Luxemburg.
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38 The secondary outcomes are:
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- 42 • Percentage of children screened (number screened/number invited)
 - 43 • Percentage of children with abnormal values
 - 44 • Percentage of children with confirmed hypercholesterolaemia and treatment
 - 45 • Percentage of families screened
 - 46 • Percentage of families with additional family members with confirmed
 - 47 hypercholesterolaemia and treatment
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55 **Ethics and Dissemination**

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57 This study has received approval from the National Research Ethics Committee in
58 Luxemburg (202108/01) and the Ministry of Health.
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3 All parents/guardians will be provided with detailed written information about the study
4 procedure. Written informed consent is necessary in order to include a child in this
5 study. All results will be published anonymously to ensure that no participant can be
6 identified. The name and identity of the participants will not appear in any of the
7 published materials.
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12 The results of this study will be disseminated in medical and scientific pre-reviewed
13 publications and conference presentations.
14
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18 19 Footnotes

- 22 • **Contributors:** MB and CdB conceived the study. MB, CdB, KW, DWD, SH,
23 FF, BZ initiated the study design and AA helped with the implementation. MB
24 and CdB are grant holders. MV, PM and VB provided statistical expertise in
25 clinical trial design. All authors contributed to refinement of the study protocol
26 and approved the final manuscript.
27
28
- 30 • **Acknowledgement:** We thank Michael Witsch who has helped to set up the
31 z-score calculation for height, bmi and blood pressure.
32
33
- 34 • **Funding statement:** This work was supported by the non-profit organization
35 “Coeur- Daniel Wagner”, grant number: N/A.
36
37
- 38 • **Competing interests statement.**
39 MB and DWD participated in a scientific advisory board of Daiichi Sankyo.
40 AA, SH FF, MV, KW, BZ, SH, PM, VC and CdB have nothing to declare.
41
42
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48 49 References

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- **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.

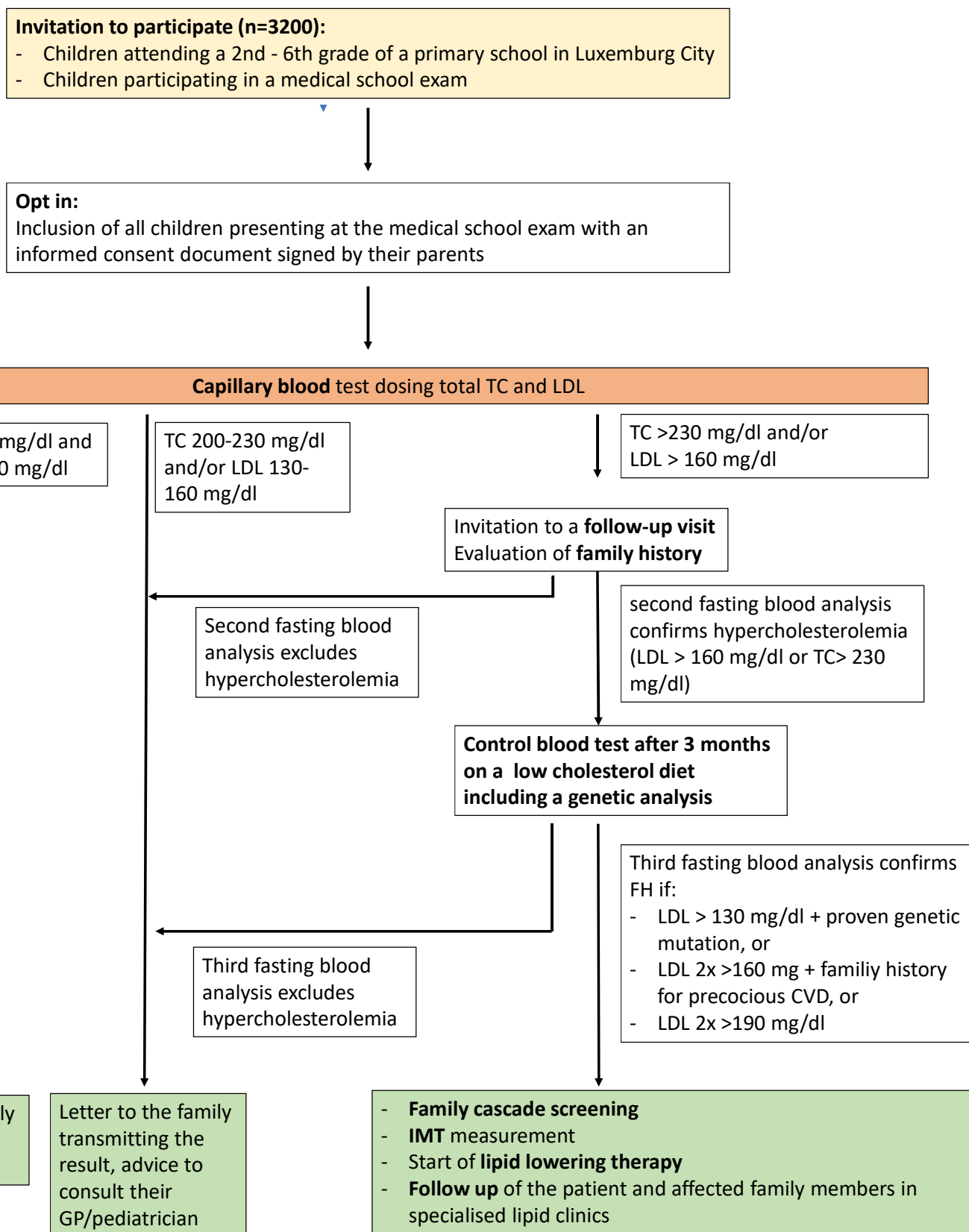


Figure 1 Study flowchart. TC: total cholesterol; LDL: low-density lipoprotein; precocious CVD: cardiovascular disease < 60 years in males/ < 55 years in females; IMT: intima media thickness; GP: general practitioner

CETTE ÉTUDE EST SOUTENUE PAR

- Direction de la Santé
- Service Médecine scolaire de la Ville de Luxembourg
- CMG Cercle des médecins généralistes
- Société Luxembourgeoise de Neurologie
- Société Luxembourgeoise de Cardiologie
- Société Luxembourgeoise de Pédiatrie

L'ÉTUDE EST FINANÇÉE PAR

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ÉTUDE PILOTE SUR LE DÉPISTAGE DE L'HYPERCHOLESTÉROLÉMIE


DIE HERZ-KREISLAUF-ERKRANKUNGEN

Herz-Kreislauf-Erkrankungen sind die Haupttodesursache in Luxemburg. Eine der möglichen Ursachen dieser Erkrankungen ist die Hypercholesterinämie (erhöhter Cholesterinwert im Blut). Oft wird dies lange Zeit nicht erkannt und nicht behandelt. Das erste Symptom ist häufig ein Herzinfarkt oder ein Schlaganfall.



FAMILIÄRE HYPERCHOLESTERINÄMIE

Die familiäre Hypercholesterinämie (FH) betrifft 1 von 250 Personen und ist eine vererbte Erkrankung (Kinder einer Person, die eine FH hat, haben ein Risiko von 50% auch betroffen zu sein). 9 von 10 Patienten wissen nicht, dass sie an einer FH leiden.




Die frühe Diagnose und Behandlung kann Leben retten!

Die Hypercholesterinämie kann durch die Untersuchung von ein paar Blutropfen diagnostiziert werden.

Wir haben daher eine Studie entwickelt, um zu prüfen, ob wir die Personen identifizieren können, die an einer familiären Hypercholesterinämie leiden und somit ein erhöhtes Risiko für Herz-Kreislauf-Erkrankungen haben.

Unsere Idee ist es, allen Kindern, die eine luxemburgische Grundschule besuchen, eine Untersuchung der Blutfette anzubieten. Wir werden in den Grundschulen der Stadt Luxemburg beginnen.


In Zusammenarbeit mit der Schulmedizin der Stadt Luxemburg wird dieses Screening im Rahmen der schulmedizinischen Untersuchung angeboten und von einem Forschungsteam der Abteilung für Kinderendokrinologie und -diabetologie des CHL (DECCP) durchgeführt.



V117.09.2021


CARDIOVASCULAR DISEASES

Cardiovascular diseases are the main cause of death in Luxembourg. One of the possible causes of these diseases is hypercholesterolaemia (elevated cholesterol level in the blood). Often this is not diagnosed or treated for a long time. Frequently, the first symptom is a heart attack or a stroke.



FAMILIAL HYPERCHOLESTEROLAEMIA

Familial hypercholesterolaemia (FH) affects 1 in 250 people and is a hereditary condition (children of a person who has FH have a 50% risk of also being affected). 9 out of 10 patients do not know that they have FH.




Early diagnosis and treatment can save lives!

Hypercholesterolaemia can be diagnosed by analysing a few drops of blood. We have therefore designed a study to see if we can identify those who have familial hypercholesterolaemia and are therefore at increased risk of cardiovascular diseases.


We propose to offer a blood lipid screening to all children attending primary schools in Luxembourg. We will start with the primary schools of Luxembourg City.

In collaboration with the school medicine of the City of Luxembourg, this screening will be offered in the setting of the medical school examination and will be carried out by a research team from the DECCP (Diabetes & Endocrinology Care Clinique Pédiatrique/ CHL).




LES MALADIES CARDIOVASCULAIRES

Les maladies cardiovasculaires sont la cause principale de décès au Luxembourg. L'une des causes possibles de ces maladies est l'hypercholestérolémie (taux élevé de cholestérol dans le sang). Elle reste souvent longtemps non détectée et donc non traitée. Le premier symptôme est souvent une crise cardiaque ou un accident vasculaire cérébral.



L'HYPERCHOLESTÉROLÉMIE FAMILIALE

L'hypercholestérolémie familiale (FH) touche 1 personne sur 250 et est une maladie héréditaire (les enfants d'une personne atteinte de FH ont un risque de 50% d'être atteints). 9 patients sur 10 ne savent pas qu'ils sont atteints d'une FH.




La détection et le traitement précoces peuvent sauver des vies!

L'hypercholestérolémie peut être diagnostiquée à travers une analyse de quelques gouttes de sang.

Nous avons développé une étude pour voir si nous pouvons identifier les personnes souffrant d'hypercholestérolémie familiale et sont donc à risque accru de maladies cardiovasculaires.


L'idée est d'offrir une analyse du bilan lipidique à tous les enfants scolarisés dans les écoles primaires au Luxembourg. Nous allons commencer par les écoles primaires de la Ville de Luxembourg.

En collaboration avec la médecine scolaire de la Ville de Luxembourg, ce dépistage sera offert lors de l'examen médical scolaire et il sera effectué par une équipe de recherche du DECCP (endocrinologie et diabétologie pédiatrique au CHL).




DOENÇAS CARDIOVASCULARES

Uma das causas possíveis destas doenças é a hipercolesterolemia (nível elevado de colesterol no sangue). Permanece frequentemente não ser detectado durante muito tempo e por isso, sem tratamento. O primeiro sintoma é frequentemente um ataque cardíaco ou um acidente vascular cerebral.



A HIPERCOLESTEROLEMIA FAMILIAR

A hipercolesterolemia familiar (FH) afecta 1 em 250 pessoas e é uma doença hereditária (cada filho de uma pessoa com FH tem 50% de probabilidade de a ter). 9 sobre 10 pacientes não têm conhecimento de que têm FH.



O diagnóstico precoce e o tratamento podem salvar vidas!

A hipercolesterolemia pode ser diagnosticada através da análise de algumas gotas de sangue.

Assim, foi desenvolvido um estudo para ver se conseguimos identificar pessoas com hipercolesterolemia familiar e, portanto, pessoas com um risco mais elevado das doenças cardiovasculares.

A ideia é oferecer um teste lipídico a todas as crianças escolarizadas nas escolas primárias do Luxemburgo.

Em colaboração com o serviço de medicina escolar da Cidade de Luxemburgo, este rastreio será oferecido durante o exame médico da escola e será levada a cabo por uma equipa de investigação do DECCP (endocrinologia pediátrica e diabétologia no CHL).




Figure 2
 Information material: Flyer in 4 languages

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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		<input type="checkbox"/> male	<input type="checkbox"/> female
weight (kg)			
height (cm)			
blood pressure (mmHg)			
Family Questionnaire		<input type="checkbox"/> yes	<input type="checkbox"/> no
if yes: high cholesterol father:	<input type="checkbox"/> yes	<input type="checkbox"/> no	<input type="checkbox"/> unknown
if yes: high cholesterol mother:	<input type="checkbox"/> yes	<input type="checkbox"/> no	<input type="checkbox"/> unknown
if yes: precocious CVD:		<input type="checkbox"/> yes	<input type="checkbox"/> no
if precocious CVD: who*:			
blood sample		<input type="checkbox"/> yes	<input type="checkbox"/> 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	<input type="checkbox"/> normal result -> result transmitted to family <input type="checkbox"/> slightly elevated -> result transmitted to family + proposition to see GP <input type="checkbox"/> abnormal result -> result transmitted to family + invitation DECCP/see GP		
<small>* GMM = grand-mère maternelle, GMP = grand-père paternel, TM = tante maternelle; TP = tante paternel CM = cousin/e maternel(le), CP = cousin/e paternel(le)</small>			

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

1			
2	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
3			n/a
4			
5			
6			(b) Report category boundaries when continuous variables were categorized
7			n/a
8			
9			(c) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period
10			n/a
11	Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses
12			n/a
13			
14	Discussion		
15	Key results	18	Summarise key results with reference to study objectives
16			n/a
17	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
18			n/a
19			
20	Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence
21			n/a
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23			
24	Generalisability	21	Discuss the generalisability (external validity) of the study results
25			n/a
26	Other information		
27	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
28			10
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*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)

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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

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

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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.

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3 **Methods and analysis** In this cross-sectional study all children (n=3200) between 7-
4 12 years, attending primary school in the city of Luxemburg, and invited for their
5 mandatory medical school exam between 2021 and 2023, are invited to participate. A
6 study nurse performs a capillary blood test to analyse the lipid profile. Families receive
7 the result including an interpretation and invitation to seek medical advice if indicated.
8 If FH is confirmed, a reverse cascade screening in that family will be proposed. The
9 child will receive standard care. Primary outcome is the occurrence of confirmed FH in
10 the study population. Secondary outcomes include the percentage of children
11 screened, percentage of children with abnormal lipid values, percentage of families
12 screened and percentage of families with additionally identified members suffering
13 from hypercholesterolaemia. A health economic analysis will be performed.

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

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33 **Study registration number:** NCT05271305; clinicaltrials.gov.

34 35 36 37 **Strengths and limitations of this study**

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

1
2
3 In primary schools, medical school examinations take place every 2 years. The families
4 of children who are invited to the medical school exam, receive written information
5 about the screening and an informed consent form. As the study is running over a
6 period of 2 years, every child will have been invited by the end of the recruitment
7 period.
8
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10
11
12 This information material includes a flyer in 4 languages (see figure 2), a flyer for
13 children adapted to their age (flyer for 7-8 years and flyer 9-12 years), and detailed
14 information about the study for the parents/caregivers.
15
16

17
18 They will receive as well a questionnaire on the family history of premature cardio-
19 cerebrovascular events and known FH disease (see figure 3).
20
21

22
23 We will promote this study in order to achieve as high a participation rate as possible
24 and in order to raise awareness for FH. Promotion will include interviews in the lay
25 press, distribution of information material to paediatricians and general practitioners,
26 teachers and the parents' representative committee. National scientific societies of
27 cardiology, neurology, paediatrics and general medicine support the study.
28
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31 32 **Patient and Public Involvement** 33

34
35 The study is in line with the demand of FH patients' groups (FH Europe), to implement
36 a paediatric screening for FH in Europe ([https://fheurope.org/policy/prague-](https://fheurope.org/policy/prague-declaration/)
37 [declaration/](https://fheurope.org/policy/prague-declaration/)). In the design of the protocol there was no patient involvement, but there
38 is patient involvement and support in the promotion of the study. Dissemination of the
39 study results is planned in scientific journals but equally in lay media in order to
40 enhance awareness of FH.
41
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44

45 46 **Study Procedures** 47

48
49 A dedicated study nurse will collect the signed informed consent forms and will perform
50 a finger prick using a Medlance® plus special blade (0,8 mm) lancet when the children
51 have their medical school exam (see figure 1).
52
53

54
55 The nurse will fill out the case record form (CRF, see supplemental material 1),
56 documenting height, weight and blood pressure measurement, data on family history
57 regarding hypercholesterolemia and precocious cardiovascular disease. All data
58 (pseudonymised) will be entered in the online database (developed by the LIH by using
59
60

1
2
3 the Vanderbilt REDcap system) for further analysis. Data will be expressed in age
4 adjusted scores.
5

6 7 ***Lipid profile measurement*** 8

9
10 The capillary blood sample (15 µl) will be analysed by the Alere Afinion 2 Analyser,
11 using the Alere Afinion™ Lipid Panel. Test result is available in 7 minutes and can be
12 printed. The Alere Afinion™ Lipid Panel includes the analysis of total cholesterol (TC),
13 high-density lipoprotein (HDL) and triglycerides by a colorimetric ELISA method. Based
14 on these results Alere Afinion 2 Analyser will calculate low-density lipoprotein (LDL). A
15 comparison between this handheld machine and CHL (Centre hospitalier de
16 Luxembourg) laboratory method (Colorimetric, enzymatic assays, Roche Cobas 8000)
17 had been conducted and demonstrated a good correlation between the TC, LDL, HDL
18 and triglyceride measurements.
19

20 21 ***Information for the families*** 22

23
24 A letter with the result of the lipid test will be sent to the family. This will include the
25 confirmation that the child has a normal result (TC < 200 mg/dl and LDL < 130 mg/dl),
26 or a recommendation to contact their doctor when the cholesterol level is slightly
27 elevated (TC 200-230 mg/dl and/or LDL 130-160 mg/dl). If cholesterol levels are high
28 (TC > 230 mg/dl and/or LDL > 160 mg/dl), the family will receive a letter with an
29 invitation for a further clinical evaluation and the advice to contact their doctor or to
30 make an appointment directly in the paediatric clinic.
31

32
33 As this first blood test is performed non-fasting, applying a calculation of the LDL levels
34 according to Friedewald, high triglyceride levels could lead to falsely elevated LDL
35 levels.
36

37 38 ***Follow-up in case of high cholesterol levels*** 39

40
41 When a high cholesterol level is detected, a detailed family history together with a
42 fasting blood test (fast for at least 8 hours) are required.
43

44
45 If the pathological values are confirmed, dietary counselling is indicated followed by a
46 fasting blood control (including TC, LDL, HDL, a genetic analysis for FH panel,
47 lipoprotein(a), serum glutamic oxaloacetic transaminase (GOT), serum glutamic
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3 pyruvic transaminase (GPT), creatine kinase (CK), thyroid-stimulating hormone (TSH))
4
5 3 months later.
6

7 Genetic analysis is coordinated and results are interpreted by the genetic department
8 of the Luxemburgish national health laboratory (Laboratoire national de santé, LNS).
9 FH panel is performed at CHU Liège and includes the following genes: *LDLR*
10 (NM_000527.4), *APOB* (NM_000384.2), *PGSK9* (NM_174936.3), *APOE* (NM-
11 a00041.3), *LDLRAP1* (NM_015627.2), *LIPA* (NM_000235.3), *ABCG5* (NM_022436.2),
12 *ABCG8* (NM_022437.2) and *STAP1* (NM_012108.3).
13
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18 FH is confirmed if[9]:
19

- 20 • genetically confirmed + LDL > 130 mg/dl
- 21 • no mutation, but 2x LDL > 190 mg/dl
- 22 • no mutation, but 2x LDL >160 mg/dl and precocious cardiovascular diseases in
23 the family
- 24
- 25
- 26
- 27
- 28

29 If FH is confirmed,
30

- 31 • cascade screening is offered to the first-degree family members (and if
32 confirmed in those to the related second-degree family members too).
- 33 • carotid intima-media thickness (cIMT) measurement before treatment will be
34 performed.
- 35 • children will be offered treatment and adult family members will be offered
36 follow-up by adult lipid specialists
- 37 • paediatric patients will be offered follow-up once every 6 months with control of
38 the lipid results and adaption of their therapy.
- 39
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46 As lipoprotein (a) is an inherited causal risk factor for the development of
47 cardiovascular disease[20], elevated lipoprotein (a) levels (> 50 mg/dl) will guide us to
48 a more aggressive lipid lowering therapy in confirmed FH cases and to initiate a lipid-
49 lowering therapy in borderline cases[21].
50
51

52 cIMT measures will be performed by the same investigator with a Siemens Acuson
53 S2000 device using a 4 to 9 MHz linear probe. Diastolic far-wall common carotid
54 intima-media thickness will be assessed proximally to the bifurcation where the
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3 vessel wall is parallel, using the cursors of the software and following the leading edge
4 system[22].
5

6
7 If indicated, children will be treated with statins, evt. if older than 10 years and
8 insufficient decrease of LDL under statin therapy is achieved, ezetimibe might be
9 added.
10
11

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13 If discordant fasting cholesterol levels are obtained (in the two fasting tests) in
14 combination with a negative family history and no genetic mutation, the patient will be
15 offered further follow up including a repeat blood test after several months.
16
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19 In case of a proven genetic mutation, genetic analysis will be offered to other affected
20 family members.
21
22

23 24 25 26 **Data management** 27

28
29 A specific electronic CRF (eCRF) is developed for the study with items related to the
30 socio-demographic characteristics of the participants and FH. CRF data will be entered
31 online in the eCRF and the data will be stored in a secured data base.
32
33

34
35 The database has been designed and the eCRF developed by the Competence Center
36 for Methodology and Statistics of LIH with a GDPR compliant data management
37 system. LIH will be responsible for the data quality control, cleaning and data analysis.
38
39

40
41 Real time Z-scores for height, BMI and blood pressure for each participant are made
42 available on the e-CRF
43
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45
46 For Z-score calculation of height and BMI, L, M and S values are taken from the WHO
47 2007 growth reference data for children and adolescents
48 (<https://www.who.int/tools/growth-reference-data-for-5to19-years/indicators>).
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52 For Z-scores of systolic and diastolic blood pressure, NHANES reference for the height
53 L, M and S values are used.
54

55
56 All personal and clinical data will be pseudonymised with an ID-number (e.g. IF0001)
57 accessible only to the dedicated employees. The delegation list of all clinical team
58 members will be kept at the DECCP and must be signed by the principal investigator.
59
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3 All access and changes in the data is tracked and monitored in an audit trail. Therefore,
4 data collection, storage and in-depth analysis respect the highest standards of data
5 protection and security.
6
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9 Only pseudonymised data without any link to personal data will be accessible by the
10 dedicated members of LIH and collaborating members and partners for analysis and
11 further investigation.
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15 16 17 **Statistical methods**

18 19 **Sample size calculation**

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21
22 The primary outcome is the prevalence of FH in children between 7-12 years in
23 Luxemburg. The sample size was calculated according to Machin and Campbell[23]
24 based on several indicators i.e. the confidence level, 1 or 2 sided interval, the expected
25 proportion, the aimed precision of the estimated proportion and the population size
26 (from which a simple random sample will be taken without replacement). As a
27 consequence, a two-sided 95% confidence interval for a single proportion using the
28 large sample normal approximation adjusted for a finite population of size of 39,000
29 (children of 7-12 years in Luxemburg) and a precision of 0.0033 for an expected
30 proportion of 0.005 of FH lead to a sample size equal to 1501.
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38 39 **Sampling plan**

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41 The sampling plan will be stratified and randomized with an allocation probability
42 (chance to be selected is equal for all individuals of the same age category and gender)
43 proportional to size (of the population) without replacement (the same individual could
44 not be selected twice). The probability to be sampled $n_h Z_{hi}$ (n_h is the sample size for
45 stratum h , and Z_{hi} is the relative size of unit i in stratum h) will be calculated for each
46 individual based on the size of each age and gender strata in the sample. It will be
47 included as a weighting parameter in the calculation of the prevalence $\sum(x_i \cdot n_h Z_{hi})$,
48 which will be estimated by summing up data on all patients. These weighting
49 estimations are elements to correct prevalence estimations in case of extreme strata
50 (undersized or oversized stratum).
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59 60 **Statistical analyses**

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3 A statistical analysis plan detailing the statistical analysis will be written blinded to the
4 data and before the end of the enrolment.
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7 A check for missing data will be performed. Several methods for processing missing
8 data are possible depending on the type of missing responses (missing completely at
9 random, missing at random or not missing at random).
10
11
12

13 The primary outcome, prevalence of FH in children between 7-12 years in Luxemburg,
14 will be calculated by summing up data on all patients and weighting by the size of each
15 age and gender strata as described earlier.
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19 To include a finite population correction in Taylor series variance estimation, the size
20 of each age and gender strata in the target population will also be entered in the
21 analyses in order to calculate the Wald 95% confidence interval, and thereby
22 extrapolate the proportion of FH to the target population. The surveyfreq procedure of
23 the statistical software SAS System V9.3 (SAS Institute, Cary, NC) will be used.
24
25
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29 To complete corrections on prevalence provided by the weighting process,
30 adjustments on age and sex are planned in order to estimate the prevalence. These
31 adjustments will also take into account discrepancies between theoretical and real
32 numbers for each stratum as well as non-responses issue.
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37 Secondary outcomes be calculated in a similar way as the primary outcome, if possible,
38 in order to enable inference to the target population of children between 7-12 years in
39 Luxemburg.
40
41
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43 The secondary outcomes are:

- 45 • Percentage of children screened (number screened/number invited)
- 46 • Percentage of children with abnormal values
- 47 • Percentage of children with confirmed hypercholesterolaemia and treatment
- 48 • Percentage of families screened
- 49 • Percentage of families with additional family members with confirmed
50 hypercholesterolaemia and treatment
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Ethics and Dissemination

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3 This study has received approval from the National Research Ethics Committee in
4 Luxembourg (202108/01) and the Ministry of Health.
5
6

7 All parents/guardians will be provided with detailed written information about the study
8 procedure. Written informed consent is necessary in order to include a child in this
9 study. All results will be published anonymously to ensure that no participant can be
10 identified. The name and identity of the participants will not appear in any of the
11 published materials.
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16 The results of this study will be disseminated in medical and scientific pre-reviewed
17 publications and conference presentations.
18
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23 Footnotes

- 24
25
26 • **Contributors:** MB and CdB conceived the study. MB, CdB, KW, DWD, SH,
27 FF, BZ initiated the study design and AA helped with the implementation. MB
28 and CdB are grant holders. MV, PM and VB provided statistical expertise in
29 clinical study design. All authors contributed to refinement of the study protocol
30 and approved the final manuscript.
31
32
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34 z-score calculation for height, bmi and blood pressure.
35
36
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39
40
- 41 • **Competing interests statement.**
42 MB and DWD participated in a scientific advisory board of Daiichi Sankyo.
43 AA, SH FF, MV, KW, BZ, SH, PM, VC and CdB have nothing to declare.
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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.

For peer review only

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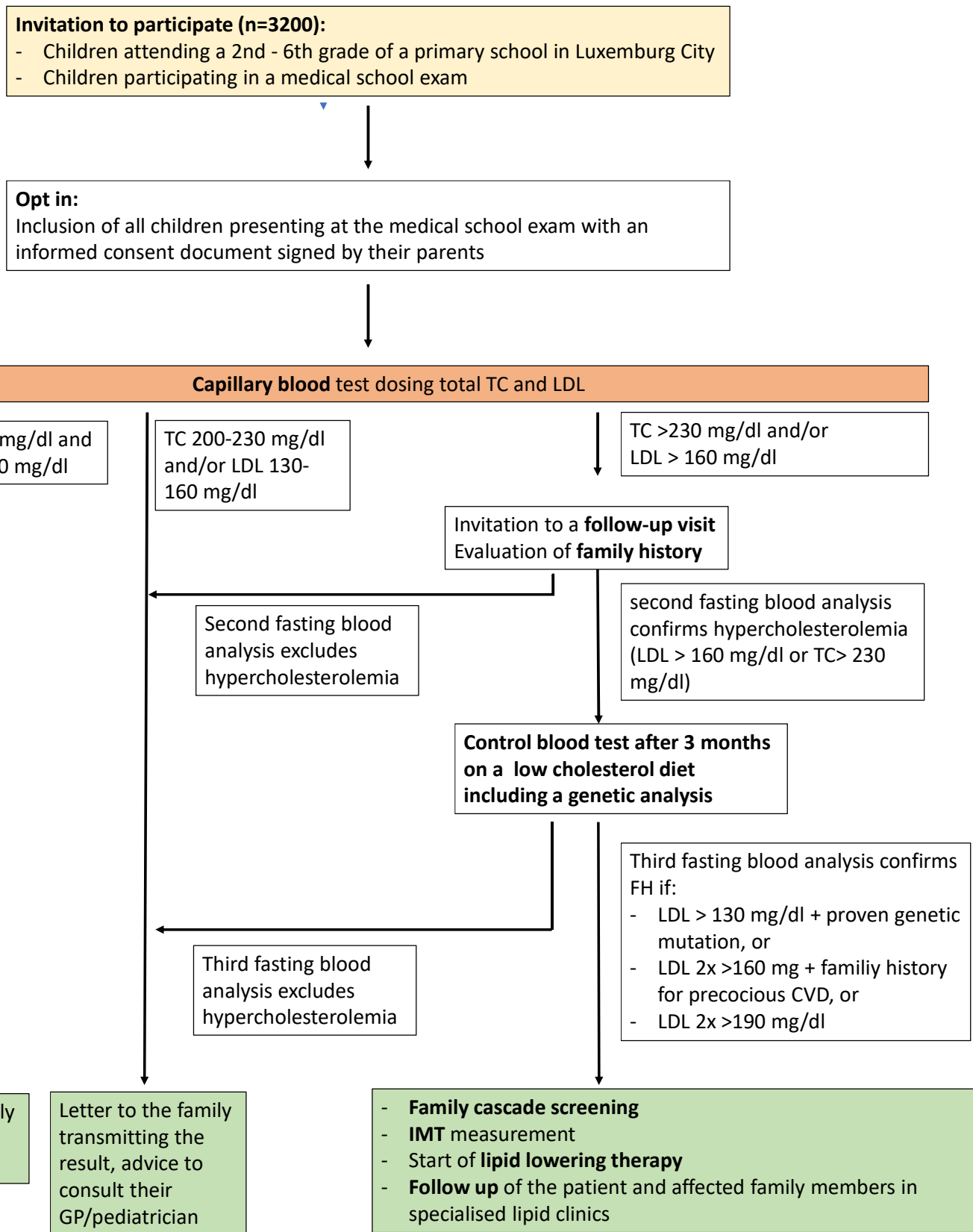


Figure 1 Study flowchart. TC: total cholesterol; LDL: low-density lipoprotein; precocious CVD: cardiovascular disease < 60 years in males/ < 55 years in females; IMT: intima media thickness; GP: general practitioner

CETTE ÉTUDE EST SOUTENUE PAR

- Direction de la Santé
- Service Médecine scolaire de la Ville de Luxembourg
- CMG Cercle des médecins généralistes
- Société Luxembourgeoise de Neurologie
- Société Luxembourgeoise de Cardiologie
- Société Luxembourgeoise de Pédiatrie

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ÉTUDE PILOTE SUR LE DÉPISTAGE DE L'HYPERCHOLESTÉROLÉMIE

LES MALADIES CARDIOVASCULAIRES

Les maladies cardiovasculaires sont la cause principale de décès au Luxembourg. L'une des causes possibles de ces maladies est l'hypercholestérolémie (taux élevé de cholestérol dans le sang). Elle reste souvent longtemps non détectée et donc non traitée. Le premier symptôme est souvent une crise cardiaque ou un accident vasculaire cérébral.

L'HYPERCHOLESTÉROLÉMIE FAMILIALE

L'hypercholestérolémie familiale (FH) touche 1 personne sur 250 et est une maladie héréditaire (les enfants d'une personne atteinte de FH ont un risque de 50% d'être atteints). 9 patients sur 10 ne savent pas qu'ils sont atteints d'une FH.

La détection et le traitement précoces peuvent sauver des vies!

L'hypercholestérolémie peut être diagnostiquée à travers une analyse de quelques gouttes de sang.

Nous avons développé une étude pour voir si nous pouvons identifier les personnes souffrant d'hypercholestérolémie familiale et sont donc à risque accru de maladies cardiovasculaires.

L'idée est d'offrir une analyse du bilan lipidique à tous les enfants scolarisés dans les écoles primaires au Luxembourg. Nous allons commencer par les écoles primaires de la Ville de Luxembourg.

En collaboration avec la médecine scolaire de la Ville de Luxembourg, ce dépistage sera offert lors de l'examen médical scolaire et il sera effectué par une équipe de recherche du DECCP (endocrinologie et diabétologie pédiatrique au CHL).

DIE HERZ-KREISLAUF-ERKRANKUNGEN

Herz-Kreislauferkrankungen sind die Haupttodesursache in Luxemburg. Eine der möglichen Ursachen dieser Erkrankungen ist die Hypercholesterinämie (erhöhter Cholesterinwert im Blut). Oft wird dies lange Zeit nicht erkannt und nicht behandelt. Das erste Symptom ist häufig ein Herzinfarkt oder ein Schlaganfall.

CARDIOVASCULAR DISEASES

Cardiovascular diseases are the main cause of death in Luxembourg. One of the possible causes of these diseases is hypercholesterolaemia (elevated cholesterol level in the blood). Often this is not diagnosed or treated for a long time. Frequently, the first symptom is a heart attack or a stroke.

DOENÇAS CARDIOVASCULARES

Uma das causas possíveis destas doenças é a hipercolesterolemia (nível elevado de colesterol no sangue). Permanece frequentemente sem ser detectado durante muito tempo e por isso, sem tratamento. O primeiro sintoma é frequentemente um ataque cardíaco ou um acidente vascular cerebral.

FAMILIÄRE HYPERCHOLESTERINÄMIE

Die familiäre Hypercholesterinämie (FH) betrifft 1 von 250 Personen und ist eine vererbare Erkrankung (Kinder einer Person, die eine FH hat, haben ein Risiko von 50% auch betroffen zu sein). 9 von 10 Patienten wissen nicht, dass sie an einer FH leiden.

FAMILIAL HYPERCHOLESTEROLAEMIA

Familial hypercholesterolaemia (FH) affects 1 in 250 people and is a hereditary condition (children of a person who has FH have a 50% risk of also being affected). 9 out of 10 patients do not know that they have FH.

A HIPERCOLESTEROLEMIA FAMILIAR

A hipercolesterolemia familiar (FH) afecta 1 em 250 pessoas e é uma doença hereditária (cada filho de uma pessoa com FH tem 50% de probabilidade de a ter). 9 sobre 10 pacientes não têm conhecimento de que têm FH.

Die frühe Diagnose und Behandlung kann Leben retten!

Die Hypercholesterinämie kann durch die Untersuchung von ein paar Blutropfen diagnostiziert werden.

Wir haben daher eine Studie entwickelt, um zu prüfen, ob wir die Personen identifizieren können, die an einer familiären Hypercholesterinämie leiden und somit ein erhöhtes Risiko für Herz-Kreislauferkrankungen haben.

Unsere Idee ist es, allen Kindern, die eine luxemburgische Grundschule besuchen, eine Untersuchung der Blutfette anzubieten. Wir werden in den Grundschulen der Stadt Luxemburg beginnen.

In Zusammenarbeit mit der Schulmedizin der Stadt Luxemburg wird dieses Screening im Rahmen der schulmedizinischen Untersuchung angeboten und von einem Forschungsteam der Abteilung für Kinderendokrinologie und -diabétologie des CHL (DECCP) durchgeführt.

Early diagnosis and treatment can save lives!

Hypercholesterolaemia can be diagnosed by analysing a few drops of blood.

We have therefore designed a study to see if we can identify those who have familial hypercholesterolaemia and are therefore at increased risk of cardiovascular diseases.

We propose to offer a blood lipid screening to all children attending primary schools in Luxembourg. We will start with the primary schools of Luxembourg City.

In collaboration with the school medicine of the City of Luxembourg, this screening will be offered in the setting of the medical school examination and will be carried out by a research team from the DECCP (Diabetes & Endocrinology Care Clinique Pédiatrique/ CHL).

O diagnóstico precoce e o tratamento podem salvar vidas!

A hipercolesterolemia pode ser diagnosticada através da análise de algumas gotas de sangue.

Assim, foi desenvolvido um estudo para ver se conseguimos identificar pessoas com hipercolesterolemia familiar e, portanto, pessoas com um risco mais elevado das doenças cardiovasculares.

A ideia é oferecer um teste lipídico a todas as crianças escolarizadas nas escolas primárias do Luxembourg.

Em colaboração com o serviço de medicina escolar da Cidade de Luxembourg, este rastreio será oferecido durante o exame médico da escola e será levada a cabo por uma equipa de investigação do DECCP (endocrinologia pediátrica e diabétologia no CHL).

Figure 2
 Information material: Flyer in 3 languages

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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		<input type="checkbox"/> male	<input type="checkbox"/> female
weight (kg)			
height (cm)			
blood pressure (mmHg)			
Family Questionnaire		<input type="checkbox"/> yes	<input type="checkbox"/> no
if yes: high cholesterol father:	<input type="checkbox"/> yes	<input type="checkbox"/> no	<input type="checkbox"/> unknown
if yes: high cholesterol mother:	<input type="checkbox"/> yes	<input type="checkbox"/> no	<input type="checkbox"/> unknown
if yes: precocious CVD:		<input type="checkbox"/> yes	<input type="checkbox"/> no
if precocious CVD: who*:			
blood sample		<input type="checkbox"/> yes	<input type="checkbox"/> 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	<input type="checkbox"/> normal result -> result transmitted to family <input type="checkbox"/> slightly elevated -> result transmitted to family + proposition to see GP <input type="checkbox"/> 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

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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.