BMJ Open

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

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

To cite: Becker M, Adamski A, Fandel F, *et al*. Screening for familial hypercholesterolaemia in primary school children: protocol for a cross-sectional, feasibility study in Luxembourg city (EARLIE). *BMJ Open* 2022;**12**:e066067. doi:10.1136/bmjopen-2022-066067

► Prepublication history and additional supplemental material for this paper are available online. To view these files, please visit the journal online (http://dx.doi.org/10.1136/bmjopen-2022-066067).

Received 03 July 2022
Accepted 20 November 2022

For numbered affiliations see end of article.

Correspondence to
Dr Marianne Becker;
becker.marianne@chl.lu

ABSTRACT

Introduction Familial hypercholesterolaemia (FH) is a frequent (1:300) autosomal dominantly inherited condition which causes premature (women <60 years, men <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 and 12 years, attending primary school in the city of Luxembourg and invited for their mandatory medical school examinations between 2021 and 2023 are invited to participate. A study nurse performs a capillary blood test to analyse the lipid profile. Families receive the result including an interpretation and invitation to seek medical advice if indicated. If FH is confirmed, a reverse cascade screening in that family will be proposed. The child will receive standard care. Primary outcome is the occurrence of confirmed FH in the study population. Secondary outcomes include the percentage of children screened, percentage of children with abnormal lipid values, percentage of families screened and percentage of families with additionally identified members suffering from hypercholesterolaemia. A health economic analysis will be performed.

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

Trial registration number NCT05271305.

STRENGTHS AND LIMITATIONS OF THIS STUDY

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

INTRODUCTION

Familial hypercholesterolaemia (FH) is an autosomal dominantly inherited genetic disorder, which causes premature arteriosclerosis leading to cardio–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 standardised 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 toddlers aged 1–2 years is cost-effective.[13] At the Technical Meeting of 2021 Slovenian EU presidency, broad professional consensus on paediatric FH screening was presented and public policy recommendations were developed.[14] Despite these facts, so far Slovenia is the only country with a national universal screening programme.[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 Luxembourg—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 healthcare systems: the cost of an universal screening per diagnosed case has been estimated at 2500 € by Wald *et al*.[13] The Luxembourg Institute of Health (LIH) estimated in 2016 the cost of a myocardial infarction survivor in Luxembourg at 15 200 € in the first year and at 2900 € for every following year. The cost for a cerebrovascular event survivor in Luxembourg was estimated at 19 500 € in the first year and 7200 € for every following year.

We hypothesise 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 into the cost-effectiveness of this screening approach.

METHODS AND ANALYSIS

Overview

Cross-sectional design, targeting all primary school children (grades 2–6) in the city of Luxembourg. The study will be performed during the mandatory medical school examination (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 Center for Methodology and Statistics.

STROBE (Strengthening the Reporting of Observational Studies in Epidemiology) 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 Luxembourg City in 2021/2022 and 2022/2023 and who are invited for the medical school examination.
- Written informed consent of the parents/caregivers.

Exclusion criteria

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

Recruitment

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

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

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

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

Patient and public involvement

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

Study procedures

A dedicated study nurse will collect the signed informed consent forms and will perform a finger prick using a Medlance plus special blade (0.8 mm) lancet when the children have their medical school examination (see figure 1).

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

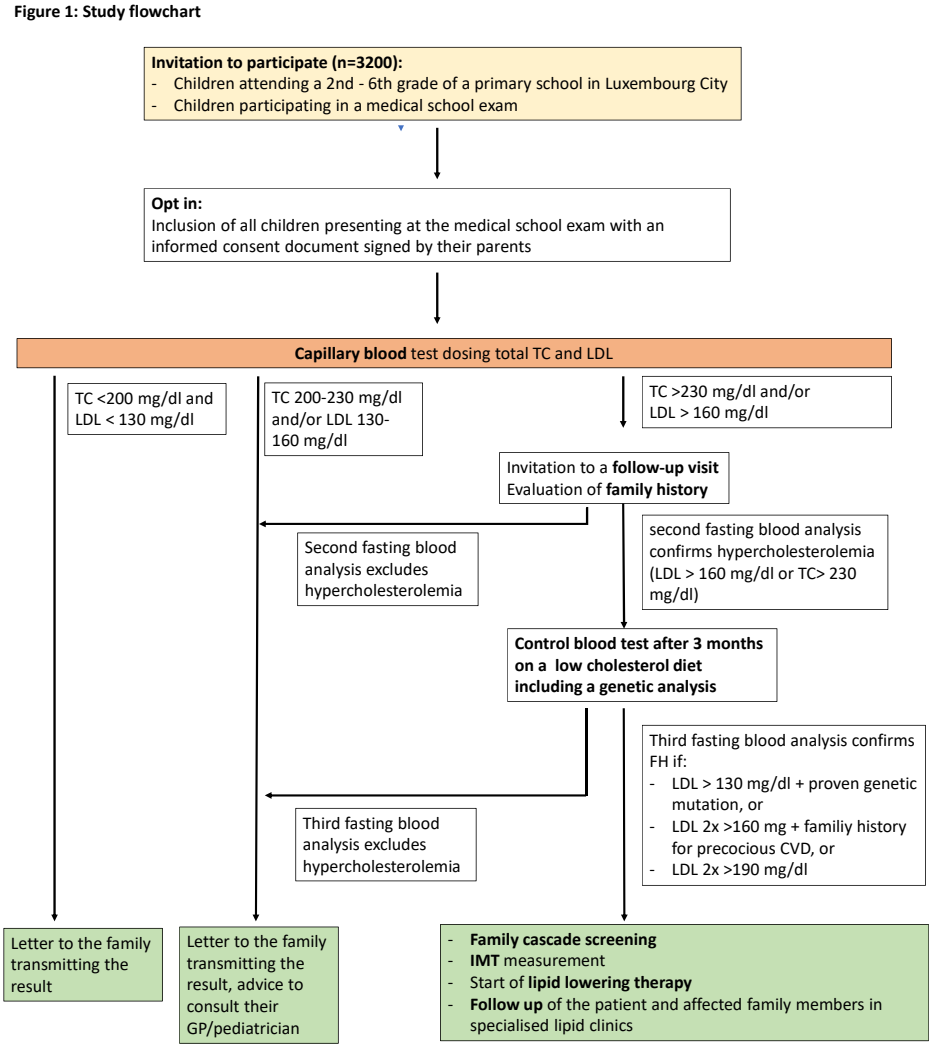

Figure 1 Study flowchart. FH, familial hypercholesterolaemia; GP, general practitioner; IMT, intima media thickness; LDL, low-density lipoprotein; precocious CVD: cardiovascular disease <60 years in men/<55 years in women; TC, total cholesterol.

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 min and can be printed. The Alere Afinion Lipid Panel includes the analysis of total cholesterol (TC), high-density lipoprotein (HDL) and triglycerides by a colorimetric ELISA method. Based on these results, Alere Afinion 2 Analyser will calculate low-density lipoprotein (LDL). A comparison between this handhold machine and CHL (Centre hospitalier de Luxembourg) laboratory method (Colorimetric, enzymatic assays, Roche Cobas 8000) had been conducted and demonstrated a good correlation between the TC, LDL, high-density lipoprotein (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

Figure 2 Information material: flyer in four languages. Reproduced with permission from Centre hospitalier de Luxembourg.

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, serum glutamic pyruvic transaminase, creatine kinase, thyroid-stimulating hormone) 3 months later.

Genetic analysis is coordinated and results are interpreted by the genetic department of the Luxembourgish 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 2× LDL >190 mg/dL.

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.

- No mutation, but 2× 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 4–9 MHz linear probe. Diastolic far-wall common carotid intima-media thickness will be assessed proximally to the bifurcation where the vessel wall is parallel, using the cursors of the software and following the leading edge system.[22]

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

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

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

Data management

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

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

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

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

For Z-scores of systolic and diastolic blood pressure, NHANES (National Health and Nutrition Examination Survey)

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

Only pseudonymised data without any link to personal data will be accessible by the dedicated members of LIH

and collaborating members and partners for analysis and further investigation.

Statistical methods

Sample size calculation

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

Sampling plan

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

Statistical analyses

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

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

The primary outcome, prevalence of FH in children between 7 and 12 years in Luxembourg, 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% CI, and thereby extrapolate the proportion of FH to the target population. The surveyfreq procedure of the statistical software SAS System V9.3 (SAS Institute) will be used.

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

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

The secondary outcomes are:

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

Ethics and dissemination

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

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

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

Author affiliations
[1]Pediatric Endocrinology and Diabetology (DECCP), Centre Hospitalier de Luxembourg, Luxembourg, Luxembourg
[2]Research Group GRON, VUB University, Brussels, Belgium
[3]Department of School Medicine of the City of Luxembourg, Luxembourg, Luxembourg
[4]Competence Center for Methodology and Statistics, Luxembourg Institute of Health, Strassen, Luxembourg
[5]Department of Paediatric Cardiology, Centre Hospitalier de Luxembourg, Luxembourg, Luxembourg
[6]Department of Neurology, Centre Hospitalier de Luxembourg, Luxembourg, Luxembourg
[7]Direction, Luxembourg Ministry of Health, Luxembourg, Luxembourg
[8]Department of Sports Medicine, Centre Hospitalier de Luxembourg, Luxembourg, Luxembourg
[9]Faculty of Science, Technology and Medicine, University of Luxembourg-Belval Campus, Esch-sur-Alzette, Luxembourg

Twitter Françoise Fandel @ff

Acknowledgements We thank Michael Witsch who has helped to set up the z-score calculation for height, BMI and blood pressure.

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

Funding This work was supported by the non-profit organisation 'Coeur-Daniel Wagner', grant number: N/A.

Competing interests MB and DWD participated in a scientific advisory board of Daiichi Sankyo. AA, SH FF, MV, KW, BZ, SH, PM, VB and CdB have nothing to declare.

Patient and public involvement Patients and/or the public were involved in the design, or conduct, or reporting, or dissemination plans of this research. Refer to the Methods section for further details.

Patient consent for publication Not applicable.

Provenance and peer review Not commissioned; externally peer reviewed.

ORCID iDs
Marianne Becker http://orcid.org/0000-0002-1643-1935
Michel Vaillant http://orcid.org/0000-0003-4714-8128
Valéry Bocquet http://orcid.org/0000-0002-3089-2877

REFERENCES

1 Austin MA, Hutter CM, Zimmern RL, *et al*. Familial hypercholesterolemia and coronary heart disease: a huge association review. *Am J Epidemiol* 2004;160:421–9.
2 Ference BA, Ginsberg HN, Graham I, *et al*. Low-Density lipoproteins cause atherosclerotic cardiovascular disease. 1. Evidence from genetic, epidemiologic, and clinical studies. A consensus statement from the European atherosclerosis Society consensus panel. *Eur Heart J* 2017;38:2459–72.
3 Pang J, Martin AC, Mori TA, *et al*. Prevalence of familial hypercholesterolemia in adolescents: potential value of universal screening? *J Pediatr* 2016;170:315–6.
4 Hu P, Dharmayat KI, Stevens CAT, *et al*. Prevalence of familial hypercholesterolemia among the general population and patients with atherosclerotic cardiovascular disease: a systematic review and meta-analysis. *Circulation* 2020;141:1742–59.
5 Beheshti SO, Madsen CM, Varbo A, *et al*. Worldwide prevalence of familial hypercholesterolemia: meta-analyses of 11 million subjects. *J Am Coll Cardiol* 2020;75:2553–66.
6 Austin MA, Hutter CM, Zimmern RL, *et al*. Genetic causes of monogenic heterozygous familial hypercholesterolemia: a huge prevalence review. *Am J Epidemiol* 2004;160:407–20.
7 , Wilemon KA, Patel J, *et al*, Representatives of the Global Familial Hypercholesterolemia Community. Reducing the clinical and public health burden of familial hypercholesterolemia: a global call to action. *JAMA Cardiol* 2020;5:217–29.
8 Mundal LJ, Igland J, Veierød MB, *et al*. Impact of age on excess risk of coronary heart disease in patients with familial hypercholesterolaemia. *Heart* 2018;104:1600–7.
9 Wiegman A, Gidding SS, Watts GF, *et al*. Familial hypercholesterolaemia in children and adolescents: gaining decades of life by optimizing detection and treatment. *Eur Heart J* 2015;36:2425–37.
10 Luirink IK, Wiegman A, Kusters DM, *et al*. 20-Year follow-up of statins in children with familial hypercholesterolemia. *N Engl J Med* 2019;381:1547–56.
11 Kusters DM, de Beaufort C, Widhalm K, *et al*. Paediatric screening for hypercholesterolaemia in Europe. *Arch Dis Child* 2012;97:272–6.
12 Vuorio A, Kuoppala J, Kovanen PT, *et al*. Statins for children with familial hypercholesterolemia. *Cochrane Database Syst Rev* 2017;7:CD006401.
13 Wald DS, Bestwick JP, Morris JK, *et al*. Child-Parent familial hypercholesterolemia screening in primary care. *N Engl J Med* 2016;375:1628–37.
14 Groselj U, Wiegman A, Gidding SS. Screening in children for familial hypercholesterolaemia: start now. *Eur Heart J* 2022;43:3209–12.
15 Groselj U, Kovac J, Sustar U, *et al*. Universal screening for familial hypercholesterolemia in children: the Slovenian model and literature review. *Atherosclerosis* 2018;277:383–91.
16 Sustar U, Kordonouri O, Mlinaric M, *et al*. Universal screening for familial hypercholesterolemia in 2 populations. *Genet Med* 2022;24:S1098-3600(22)00819-X:2103–11.
17 Scharel L. *Statistiques des causes de décès pour l'année*. 84, 2019.
18 Global health estimates: life expectancy and leading causes of death and disability. Available: https://www.who.int/data/gho/data/themes/mortality-and-global-health-estimates [Accessed 26 Feb 2022].
19 von Elm E, Altman DG, Egger M, *et al*. The strengthening the reporting of observational studies in epidemiology (STROBE) statement: guidelines for reporting observational studies. *Lancet* 2007;370:1453–7.
20 Welsh P, Welsh C, Celis-Morales CA, *et al*. Lipoprotein(a) and cardiovascular disease: prediction, attributable risk fraction, and estimating benefits from novel interventions. *Eur J Prev Cardiol* 2022;28:1991–2000.
21 Catapano AL, Daccord M, Damato E, *et al*. How should public health recommendations address Lp(a) measurement, a causative risk factor for cardiovascular disease (CVD)? *Atherosclerosis* 2022;349:136–43.
22 Droste DW, Schulte V, Schomacher G, *et al*. Comparison of automatic intima media thickness measurement and eye balling. *Cerebrovasc Dis* 2010;29:307–8.
23 Machin D, Campbell MJ. *Statistical tables for the design of clinical trials*. Illustrated edition. Oxford England ; Boston : St Louis, Mo: Blackwell Scientific Publications, 1987.