PEER REVIEW HISTORY

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ARTICLE DETAILS

TITLE (PROVISIONAL)	Screening for familial hypercholesterolaemia in primary school children: protocol for a cross-sectional, feasibility study in Luxemburg city (EARLIE)
AUTHORS	Becker, Marianne; Adamski, Aurélie; Fandel, Françoise; Vaillant, Michel; Wagner, Kerstin; Droste, Dirk; Ziade, Bechara; Hein, Steve; Mendon, Priyanka; Bocquet, Valéry; de Beaufort, Carine

VERSION 1 – REVIEW

REVIEWER	Pang, Jing University of Western Australia, School of Medicine and Pharmacology
REVIEW RETURNED	22-Jul-2022

GENERAL COMMENTS	This is a protocol paper on the screening of primary school aged children for FH in Luxemburg city. FH diagnosis and treatment in early life is important and such a screening program would be of great benefit. However, the paper is not particularly well written. Major revisions and more thought required. Abstract- -what is meant by “precocious cerebral-cardio vascular disease”? cardiovascular should be one word -an adjacent cascade screening “program”? -comma after “In this cross-sectional study” -capillary blood test for what? Introduction- -there are now more updated references for prevalence, eg. Hu P, Dharmayat KI, Stevens CAT, Sharabiani MTA, Jones RS, Watts GF, 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. Beheshti SO, Madsen CM, Varbo A, Nordestgaard BG. Worldwide Prevalence of Familial Hypercholesterolemia: Meta-Analyses of 11 Million Subjects. J Am Coll Cardiol. 2020;75:2553-66. Or more relevant, there is a prevalence study in children: Pang J, Martin AC, Mori TA, Beilin LJ, Watts GF. Prevalence of familial hypercholesterolemia in adolescents: potential value of universal screening?. The Journal of pediatrics. 2016;170:315-6. -the paragraph on costs can be succinctly framed regarding the potential cost savings of this screening program Methods-

	-in the second year, will you only need to screen children that moved from Grade 1 into Grade 2, and those that did not consent the previous year to avoid double up? Also there is so much repetition re the 2021/2022 and 2022/2023 school years, this is unnecessary -will the national paediatric clinic have the resources to take on the potential onslaught of referrals? -how will the study be promoted among parents? -I do not understand the patient involvement section -"she" – will the dedicated study nurse always be female? -please reference the capillary blood test methodology -total cholesterol is generally abbreviated as TC, not CT -sometimes LDL, LDLC, LDL cholesterol – be consistent! -what is a fasting blood control? Also spell out abbreviations for the tests -Given lipoprotein(a) will be measured, what is the protocol for those with both elevated lipoprotein(a) and FH? will there be cascade testing for Lp(a) offered? -remove "a" before cascade screening -spell out cIMT, is there a protocol for this and how the results will be used? -remove "a" before treatment, and before follow-up -potentially, what treatment will these children be offered? -please provide a copy of the CRF as supplementary material, spell out CRF, details of the CRF can also be in the supplementary material eg. real time scores, alerts, validation etc -I do not follow the power calculation and sampling plan section -regarding the prevalence calculation, would it not be better to do an age standardised prevalence and report with 95% CI? General- -please flesh out more limitations of this study -would there be an opportunity to collection qualitative or semiqualitative information on the experience of this screening program for the children and parents?

REVIEWER	Wiegman, Albert Amsterdam UMC Locatie AMC Vrouw- KindCentrum, pediatrics
REVIEW RETURNED	09-Aug-2022

GENERAL COMMENTS	An interesting topic for And a pilot study And a city-wide screening at the same time, because FH is a frequent disorder worldwide, and Luxembourg will be able to attract many children between 7 and 12, because of their health system and their dedicated specialists. I have some minor comments: In the Introduction, the authors refer to a paper of the year 1991 [ref 4], whereas a more recent paper from Mundal in Heart 2018 is available, who downwards revised the expected numbers of CHD, with a standardized incidence ratio between age 25 and 40 of 11.1 for men and 17.3 for women (higher in women, because the chance on CHD for women between 25 and 40 in normal population is extremely low) [Mundal LJ e.a. Heart 2018;104:1600-7]. In the Methods and Analysis the authors schedule three blood tests for the group of children with highest cholesterol levels. Why is this not restricted to two blood controls (including (potential)

	genetic analysis etc, only performed when the second cholesterol measurement is also high)? After two times high cholesterol levels, the diagnosis is confirmed (see the text in their paragraph: Follow-up in case of high cholesterol levels). Every extra blood puncture in itself can cause withdrawal from the study. And what about confusing results of for instance twice LDL-c > 190mg/dL and once LDL-c of 155 mg/dL? What will the authors decide? And why do the authors test total cholesterol (TC), while their diagnostic decisions are made only on LDL-c? If TC is in the upper range of 200-230 mg/dL, LDL-c can still exceed the 160 mg/dL. So, what can TC perhaps bring other than confusion, if they won't perform LDL-c measuring in every child? In their Questionnaire for parents in the setting of the hypercholesterolemia screening study, the authors ask for elevated cholesterols in the parent and for receiving medication for that. Do the authors include the outcome of this question in the final decision making? In ref 6 [Eur Heart J 2015 Wiegman e.a. (flowchart fig. 6)], high LDL-c in a parent in combination with LDL-c > 160 mg/dL in a child also leads to 'confirmed FH' (and if baseline LDL-c was unknown, the receiving of lipid lowering treatment is a good alternative). At yet, it is uncertain what the authors will do, because it is not written in the text, nor in the study flowchart (fig 1). Finally a suggestion: to add to the second question a third option: I don't know my family history. Although rare, it does occur. I whish the authors with their EARLIE study lots of success and many interesting results.

REVIEWER	Groselj, Urh UMC - University Children's Hospital Ljubljana, Dept. of Pediatric Endocrinology, Diabetes and Metabolism
REVIEW RETURNED	18-Aug-2022

GENERAL COMMENTS	This is a very interesting study reporting a protocol of a pilot project on familial hypercholesterolemia screening program in Luxemburg. Despite the paper is conceptual at this phase it brings very important data, which might be of a wide professional interest. The study design addresses several important issues (e.g. the uptake of opt-in program; the cost-effectiveness of this approach; use of capilary test) and is meticulously designed. it presents the protocol and materials the authors have developed also based on some previously reported programs. I belive it thus merits publication, also to help raising awareness on this important issue. Outcomes are clearly defined. Several minor aspects could be addressed: - throughout the manuscript, some statements (Introduction, Discussion) or some parts of the study protocol are not substantiated with references (e.g. diagnostic criteria for FH etc) - please address it. - would be important to further elaborate on the strategy of genetic testing for FH, which is planned (e.g. where, how, what will be in panel, how the variants will be interpreted/classified, genetic testing of parents/siblings etc). - there was one very recent report on opt-in pilot uni screening study in Lower Saxony (DOI: 10.1016/j.gim.2022.06.010) - might be good to mention it in this context.

	- might be good to Discuss some aspects of protocol with reviewing the relevant literature to put them in the uptodate context (if the format of paper allows it) - especially those aspects where the study provides novel perspective on this issue (e.g. use of capillary blood tests; opt-in approach; healt-economics of universal screening for FH etc) - once the abbr. is established (e.g. FH) it should be used later on consistently - please revisit.

VERSION 1 – AUTHOR RESPONSE

Reviewer: 1
Dr. Jing Pang, University of Western Australia
Comments to the Author:
This is a protocol paper on the screening of primary school aged children for FH in Luxemburg city. FH diagnosis and treatment in early life is important and such a screening program would be of great benefit. However, the paper is not particularly well written. Major revisions and more thought required.
Thank you very much for your thorough and detailed review of our manuscript. We modified accordingly and we hope that the manuscript has improved substantially.

Abstract-
-what is meant by “precocious cerebral-cardio vascular disease”? cardiovascular should be one word
We corrected the spelling mistake and defined further by adding the age details: ”which causes **premature (female < 60 years, male < 55 years)** *cardio-cerebrovascular disease (CVD)* ~~*in early adulthood*~~*.”*
-an adjacent cascade screening “program”?
Sorry, but we could not find the cited “program”, so we were not quite sure, how to improve the sentence: “a Reverse cascade screening targets to detect other affected family members.”
-comma after “In this cross-sectional study”
Thank you, we added the comma.
-capillary blood test for what?
We added an explanation:
*“**A study nurse performs a capillary blood test to analyse the lipid profile**.”*

Introduction-
-there are now more updated references for prevalence, eg.
Hu P, Dharmayat KI, Stevens CAT, Sharabiani MTA, Jones RS, Watts GF, 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.
Beheshti SO, Madsen CM, Varbo A, Nordestgaard BG. Worldwide Prevalence of Familial Hypercholesterolemia: Meta-Analyses of 11 Million Subjects. J Am Coll Cardiol. 2020;75:2553-66.
Or more relevant, there is a prevalence study in children:
Pang J, Martin AC, Mori TA, Beilin LJ, Watts GF. Prevalence of familial hypercholesterolemia in adolescents: potential value of universal screening?. The Journal of pediatrics. 2016;170:315-6.
Thank you very much, we updated the references and corrected the prevalence estimation accordingly.
-the paragraph on costs can be succinctly framed regarding the potential cost savings of this screening program
We consider the country specific cost calculation important for the planned analysis of cost-effectiveness and requested these cost estimations performed by the Luxembourg Institute of Health. In our opinion, these numbers illustrate clearly the economic impact of such a screening. We would hence prefer not to shorten this paragraph.

Methods-
-in the second year, will you only need to screen children that moved from Grade 1 into Grade 2, and those that did not consent the previous year to avoid double up?

The medical school exam is performed every 2nd year, so at grade 2, 4 and 6. So indeed we will invite the children who moved from grade 1 to grade 2, but as the invitation will be send out only for every 2nd grade, there will be no risk of double invitations.
Thank you for making this point, we changed the manuscript accordingly:
"In primary schools, medical school examinations take place every 2 years. The families of children who are invited to the medical school exam, receive written information about the screening and an informed consent form. As the study is running over a period of 2 years, every child will have been invited by the end of the recruitment period."

Also there is so much repetition re the 2021/2022 and 2022/2023 school years, this is unnecessary
We shortened the text to avoid these repetitions.

-will the national paediatric clinic have the resources to take on the potential onslaught of referrals?
Yes. As the referral will only concern children with high cholesterol levels, this will be the case for about 10-20 children over a period of 2 years.

-how will the study be promoted among parents?
The families receive written information (a flyer and a more detailed information), the study has been reported on the national television program and in two magazines, pediatricians and general practitioners have been informed and received posters to put up in their cabinets. On all these information materials, we have printed the contact details of the PIs. This will render it easy for families to contact the team for more information
We also discussed the study with parents' representatives of the school council and informed the teachers so that they can pass on information about the study to parents during parents' reunions.
We added the following sentence to the manuscript:
***"*Promotion will include interviews in the lay press, distribution of information material to paediatricians and general practitioners, to teachers and to the parents' representative committee."**

-I do not understand the patient involvement section
We rephrased the patient involvement section in order to make it more comprehensible:
"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."

-"she" – will the dedicated study nurse always be female?
Thank you for pointing this out, we absolutely agree and rephrased.

-please reference the capillary blood test methodology
We detailed the capillary blood test, by adding information about the lancet:
"will perform a finger prick **using a Medlance® plus special blade (0,8 mm) lancet***."*
The methodology for the laboratory is referenced under " Lipid profile measurement" (The Alere Afinion™ Lipid Panel includes the analysis of total cholesterol, HDL and triglycerides by a colorimetric ELISA method).

-total cholesterol is generally abbreviated as TC, not CT
We adapted the abbreviation according to your suggestion, thank you.

-sometimes LDL, LDLC, LDL cholesterol – be consistent!
We adapted our text and used "LDL" as abbreviation throughout the whole manuscript.

-what is a fasting blood control?
We added a definition: "a fasting blood test **(fast for at least 8 hours)***".*

Also spell out abbreviations for the tests
*We spelt out the abbreviations: "*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)".

-Given lipoprotein(a) will be measured, what is the protocol for those with both elevated lipoprotein(a) and FH?
We added the ratio and our protocol for Lp(a) analysis:
"As lipoprotein (a) is an inherited causal risk factor for the development of cardiovascular disease [16], 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 [17]."

- will there be cascade testing for Lp(a) offered?
Lip(a) will be included in the cascade screening blood test for first degree relatives, but as this is a screening targeting to detect FH, we will not perform cascade screening, if a child has normal lipid levels (TC, LDL) but elevated Lp(a). Nevertheless, we will inform the family about the result explaining, that Lp(a) is an inherited risk factor and if they wish to do a blood test, we will of course prescribe this for the first-degree family members.

-remove "a" before cascade screening
We removed it.

-spell out cIMT, is there a protocol for this and how the results will be used?
We spelled out and added a protocol for the measurements:
"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 [18]."

-remove "a" before treatment, and before follow-up
We removed it.

-potentially, what treatment will these children be offered?
We specified the treatment: **"If indicated, children will be treated with statins, evt. if older than 10 years and insufficient decrease of LDL under statin therapy is achieved, ezetimibe might be added."**

-please provide a copy of the CRF as supplementary material, spell out CRF, details of the CRF can also be in the supplementary material eg. real time scores, alerts, validation etc
We spelled out CRF (in the study procedure section), provided a CRF copy as supplementary material and transferred the details of the eCRF to the supplementary material (see supplement 1).

-I do not follow the power calculation and sampling plan section
We detailed this paragraph to make it clearer:
"The primary outcome is the prevalence of FH in children between 7-12 years in Luxemburg. The sample size was calculated according to Machin and Campbell [19] based on several indicators i.e. the confidence level, 1 or 2 sided interval, the expected proportion, the aimed precision of the estimated proportion and the population size (from which a simple random sample will be taken without replacement). As a consequence, a two-sided 95% confidence interval for a single proportion using the large sample normal approximation adjusted for a finite population of size of 39,000 (children of 7-12 years in Luxemburg) and a precision of 0.0033 for an expected proportion of 0.005 of FH lead to a sample size equal to 1501."

-regarding the prevalence calculation, would it not be better to do an age standardised prevalence and report with 95% CI?
We tried to make this paragraph clearer in adding some precisions.
We agree with you when you mention the utility of an adjustment to correct an estimated prevalence. However, it is necessary to use before all a process of weightings. Indeed, before data collection, it is necessary to calculate weightings associated to each stratum from the population size in each stratum. These weighting estimations are elements to correct prevalence estimations in case of extreme strata (undersized or oversized stratum). A future adjustment would not be enough to correct

these discrepancies. However, as you say, in spite of everybody's good will, the number of people in each stratum could differ from estimations provided in a protocol. It the moment, where adjustments could marginally correct estimations obtained, taking into account also non-responses.
We added details in the sampling plan:
"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)."**
And added the following sentence in the statistical analysis section:
"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."

General-
-please flesh out more limitations of this study
We added the 2 following limitations:
"- 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."

-would there be an opportunity to collection qualitative or semiqualitative information on the experience of this screening program for the children and parents?
This is a very interesting point and we will discuss it in our study committee.

Reviewer: 2
Dr. Albert Wiegman, Amsterdam UMC Locatie AMC Vrouw- KindCentrum
Comments to the Author:
An interesting topic for And a pilot study And a city-wide screening at the same time, because FH is a frequent disorder worldwide, and Luxembourg will be able to attract many children between 7 and 12, because of their health system and their dedicated specialists.

I have some minor comments:

In the Introduction, the authors refer to a paper of the year 1991 [ref 4], whereas a more recent paper from Mundal in Heart 2018 is available, who downwards revised the expected numbers of CHD, with a standardized incidence ratio between age 25 and 40 of 11.1 for men and 17.3 for women (higher in women, because the chance on CHD for women between 25 and 40 in normal population is extremely low) [Mundal LJ e.a. Heart 2018;104:1600-7].
Thank you, we updated the reference and adapted our manuscript accordingly.

In the Methods and Analysis the authors schedule three blood tests for the group of children with highest cholesterol levels. Why is this not restricted to two blood controls (including (potential) genetic analysis etc, only performed when the second cholesterol measurement is also high)? After two times high cholesterol levels, the diagnosis is confirmed (see the text in their paragraph: Follow-up in case of high cholesterol levels). Every extra blood puncture in itself can cause withdrawal from the study.
As the first blood test is performed at school in a non-fasting situation and the results of the handhold device are based on a calculation of LDL according to Friedewald, which could be altered by high triglyceride levels, we decided to do first a confirmatory fasting blood test. Following guidelines, if high cholesterol levels are confirmed, there will be a dietary intervention, and the second blood test (including the genetic analysis) will be performed fasting and under a low cholesterol diet.
We will evaluate after the study the number of withdrawals of patients and will analyse whether the results of the non-fasting calculated and the fasting measured LDL values differ significantly. As we hope to implement a nationwide screening after this pilot study these points will be carefully evaluated to keep the blood tests to the absolute minimum.

We added a sentence in order to explain our reasoning:

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

and we added this procedure as limitation of the study:
“Confirmation by fasting venous blood samples might decrease the participation rate.”

And what about confusing results of for instance twice LDL-c > 190mg/dL and once LDL-c of 155 mg/dL? What will the authors decide?
The three blood samples will be performed under different conditions: first non-fasting calculated according to Friedewald, second fasting, third fasting and under low cholesterol diet, so the third blood result should be the most reliable one.
Family history is another important factor to take into account (as described in our definition of FH). As the third blood tests will include a genetic analysis, we will have an additional information to diagnose or not FH.
If a child (following your example) would have a first fasting blood test of 190 mg/dl and a second of 155 mg/dl with no mutation detected (as otherwise he/she would already classify as FH) with no family history but with an obesity, we would work on his/her weight and follow up on the LDL development. If the same child is non-obese but has a strong family history for precocious CVD, we will perform another blood test or if on top Lp(a) is very elevated, start a lipid-lowering therapy.
So, in conclusion, we are aware that there will be confusing results and we will certainly follow them up further and will have to decide on a case-to-case base including additional factors on how to classify and treat them.
We added the following sentence to address this difficult situation:
“If discordant fasting cholesterol levels are obtained (in the two fasting tests) in combination with a negative a family history and no genetic mutation, the patient will be offered further follow up including a repeat blood test after several months.”

And why do the authors test total cholesterol (TC), while their diagnostic decisions are made only on LDL-c? If TC is in the upper range of 200-230 mg/dL, LDL-c can still exceed the 160 mg/dL. So, what can TC perhaps bring other than confusion, if they won't perform LDL-c measuring in every child?
We totally agree with the reviewer. Fact is, that the applied lipid panel automatically provides TC, HDL and LDL results and these results are displayed as well on the printout which is sent to the families. So we included TC in the protocol, but after the first year, we had some patients with high TC and HDL values, who were detected by the screening (although normal LDL). But these findings are exactly, what we wanted to collect in our pilot study in order to plan a nationwide screening program in the best possible way.

In their Questionnaire for parents in the setting of the hypercholesterolemia screening study, the authors ask for elevated cholesterols in the parent and for receiving medication for that. Do the authors include the outcome of this question in the final decision making? In ref 6 [Eur Heart J 2015 Wiegman e.a. (flowchart fig. 6)], high LDL-c in a parent in combination with LDL-c > 160 mg/dL in a child also leads to 'confirmed FH' (and if baseline LDL-c was unknown, the receiving of lipid lowering treatment is a good alternative). At yet, it is uncertain what the authors will do, because it is not written in the text, nor in the study flowchart (fig 1).
We had defined in our text the diagnosis of FH based on the flowchart of the cited publication:
Familial hypercholesterolaemia is confirmed if [9]:
- *genetically confirmed + LDL cholesterol > 130 mg/dl*
- *no mutation, but 2x LDL cholesterol > 190 mg/dl*
- *no mutation, but 2x LDL cholesterol >160 mg/dl and precocious cardiovascular diseases in the family*

We added this definition as well to the flowchart (see figure 1).

Finally a suggestion: to add to the second question a third option: I don't know my family history. Although rare, it does occur.
Thank you for this advice, we will adapt the questionnaire, but this has to be approved by our local Ethical Committee, so we can not apply the changes right away.

I whish the authors with their EARLIE study lots of success and many interesting results.
Thank you so much!

Reviewer: 3
Dr. Urh Groselj, UMC - University Children's Hospital Ljubljana
Comments to the Author:
This is a very interesting study reporting a protocol of a pilot project on familial hypercholesterolemia screening program in Luxemburg. Despite the paper is conceptual at this phase it brings very important data, which might be of a wide professional interest. The study design addresses several important issues (e.g. the uptake of opt-in program; the cost-effectiveness of this approach; use of capilary test) and is meticulously designed. it presents the protocol and materials the authors have developed also based on some previously reported programs. I believe it thus merits publication, also to help raising awareness on this important issue. Outcomes are clearly defined.

Several minor aspects could be addressed:
- throughout the manuscript, some statements (Introduction, Discussion) or some parts of the study protocol are not substantiated with references (e.g. diagnostic criteria for FH etc) - please address it.
We added the following references:

- Familial hypercholesterolaemia (FH) is an autosomal dominantly inherited genetic disorder, which causes premature arteriosclerosis leading to cardio- and cerebrovascular disease **[1,2].**
- Mutations are often found in the low-density lipoprotein receptor gene (LDLr), apolipoprotein B gene (APOB), or the proprotein convertase subtilisin/kexin type 9 gene (PCSK9**)[6].**
- Familial hypercholesterolaemia is confirmed if **[9]**:

- would be important to further elaborate on the strategy of genetic testing for FH, which is planned (e.g. where, how, what will be in panel, how the variants will be interpreted/classified, genetic testing of parents/siblings etc).
We elaborated further on the genetic testing strategy:
"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)."
And regarding testing of family members we added the following sentence:
"In case of a proven genetic mutation, a genetic analysis will be offered to other affected family members too."

- there was one very recent report on opt-in pilot uni screening study in Lower Saxony (DOI: 10.1016/j.gim.2022.06.010) - might be good to mention it in this context.
Thank you we added this reference:
"An opt-out approach – as applied in the Slovenian screening program- would very likely result in a higher participation rate [17], but due to ethical restrictions, only an opt-in approach was feasible for this study."

- might be good to Discuss some aspects of protocol with reviewing the relevant literature to put them in the uptodate context (if the format of paper allows it) - especially those aspects where the study provides novel perspective on this issue (e.g. use of capillary blood tests; opt-in approach; healt-economics of universal screening for FH etc)
Thanks a lot for these excellent points. Based on the format of this paper ("protocol") we did not foresee this discussion. These points will certainly be addressed when reporting the results of our study.

- once the abbr. is established (e.g. FH) it should be used later on consistently - please revisit.
Thank you, we revisited our manuscript accordingly (FH, TC).

Reviewer: 1
Competing interests of Reviewer: None

Reviewer: 2
Competing interests of Reviewer: No competing interests

Reviewer: 3
Competing interests of Reviewer: None.

VERSION 2 – REVIEW

REVIEWER	Wiegman, Albert Amsterdam UMC Locatie AMC Vrouw- KindCentrum, pediatrics
REVIEW RETURNED	11-Oct-2022

GENERAL COMMENTS	Excellent, the way you reply to all peer reviewers. It improved the manuscript and will also improve the clearity of the outcome of the investigation