PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	Prospective cohort study of genomic newborn screening: BabyScreen+ pilot study protocol
AUTHORS	Lunke, Sebastian; Bouffler, Sophie; Downie, Lilian; Caruana, Jade; Amor, David J.; Archibald, Alison; Bombard, Yvonne; Christodoulou, John; Clausen, Marc; De Fazio, Paul; Greaves, Ronda; Hollizeck, Sebastian; Kanga-Parabia, Anaita; Lang, Nitzan; Lynch, Fiona; Peters, Riccarda; Sadedin, Simon; Tutty, Erin; Eggers, Stefanie; Lee, Crystle; Wall, Meaghan; Yeung, Alison; Gaff, Clara; Gyngell, Christopher; Vears, Danya F; Best, Stephanie; Goranitis, I; Stark, Zornitzaa

VERSION 1 – REVIEW

REVIEWER	Hristova-Atanasova, Eleonora Medical University of Plovdiv, Social medicine and Public Health
REVIEW RETURNED	13-Nov-2023

GENERAL COMMENTS	The title, abstract and keywords accurately reflect the content of the manuscript and underlying data. The work includes 29 references. My recommendation is to cite more research articles from the last 2 years. Sikonja, J.; Groselj, U.; Scarpa, M.; la Marca, G.; Cheillan, D.; Kölker, S.; Zetterström, R.H.; Kožich, V.; Le Cam, Y.; Gumus, G.; et al. Towards Achieving Equity and Innovation in Newborn Screening across Europe. Int. J. Neonatal Screen. 2022, 8, 31. Koracin, V.; Mlinaric, M.; Baric, I.; Brincat, I.; Djordjevic, M.; Torkar, A.D.; Fumic, K.; Kocova, M.; Milenkovic, T.; Moldovanu, F.; et al. Current Status of Newborn Screening in Southeastern Europe. Front. Pediatr. 2021, 9, 648939. Spiekerkoetter, U.; Bick, D.; Scott, R.; Hopkins, H.; Krones, T.; Gross, E.S.; Bonham, J.R. Genomic newborn screening: Are we entering a new era of screening? J. Inherit. Metab. Dis. 2023, 46, 778–795. Iskrov, G.; Angelova, V.; Bochev, B.; Valchinova, V.; Gencheva, T.; Dzhuleva, D.; Dichev, J.; Nedkova, T.; Palkova, M.; Tyutyukova, A.; et al. Prospects for Expansion of Universal Newborn Screening in Bulgaria: A Survey among Medical Professionals. Int. J. Neonatal Screen. 2023, 9, 57. And still others... The author must systematically present and discuss the results of the research tasks and make detailed discussion and conclusion parts. The author must summarize the conclusion and correspond to the tasks of the topic.

REVIEWER	Hammarström , Lennart Karolinska Institute

REVIEW RETURNED	21-Nov-2023

GENERAL COMMENTS	A straight forward protocol for genomic screening of 1000 newborn babies and considering "Australia" specific aspects of this type of studies. There are some additional studies already ongoing/published which might be added to the publication list, allowing comparison.

REVIEWER	Han , Lianshu Shanghai Jiao Tong University School of Medicine
REVIEW RETURNED	28-Nov-2023

GENERAL COMMENTS	Authors in "Prospective cohort study of genomic newborn screening: BabyScreen+ study protocol" provided a three-phase process (pre-implementation, implementation and post-implementation phases) for the evaluation of the feasibility of gNBS in Australia. The pre- and post-test processes were designed in great detail: the application of the online recruitment tools will facilitate education and consent of participants; multi-stage surveys and interviews were beneficial in assessing the acceptance and opinions of parents, HCP, and the Australian public about the program. However, the design of the implementation phase was not comprehensive enough, particularly in terms of technology assessment. 1.As the authors mentioned (see “Study timetable and sites”), the pre-implementation phase of this project has been underway for a year. The details about the preparation of the materials (Page 9, lines 52-58) were suggested to be presented in the supplementary materials rather than stated in outline form. Screened conditions and selected genes included in the study should be listed in the supplementary materials. 2.The interval between the birth of the child and the receipt of the test results should be as short as possible to ensure that results are returned within a clinically meaningful timeframe. Current research has been able to reduce this time interval to less than 20 days (PMID: 37656460). The time lag between enrollment and results in this program is up to 1.5 months (enrollment: 2 weeks after birth + results return: 4 weeks after sample collection), which is detrimental to the timely diagnosis and treatment of diseases (especially some genetic metabolic diseases). 3.Does the participants pay for the gNBS? This may be an important factor in parental acceptance of the program acceptability of parents and the public. 4.Methods and analytical processes regarding sequencing need to be described in more detail, and references for analytical methods need to be cited rather than just the manufacturer. 5.The value of gNBS is not fully exploited. Only variants that are classified as likely pathogenic or pathogenic will be reported and other information (carrier status, adult-onset conditions, or variants of uncertain significance) will be ignored, which will greatly affect the sensitivity of the test and result in compromised costs and benefits. It is recommended that a long-term follow-up process be added so that sequencing data can be reanalyzed when subjects develop disease phenotypes. The clinical significance of variants of uncertain significance (VUSs) in genes related to the existing phenotype and

	genes with moderate or limited evidence of causing the specific indication, will be re-evaluated based on indications, further segregation analysis and clinical evaluation. Furthermore, carrier-status information is helpful in future reproductive planning at a time when families are having children. 6.What does stdNBS refer to? Is it tandem mass spectrometry screening? 7.The authors mentioned in the “secondary aims” to compare the performance of stdNBS and gNBS, which is critical for evaluating the diagnostic performance and clinical utility of gNBS. Authors need to describe the comparison process and indicators in detail. Minor issue: “stdNBS” and REDCap should be labeled with their full names at the time of initial appearance.

VERSION 1 – AUTHOR RESPONSE

Reviewer: 1
Dr. Eleonora Hristova-Atanasova, Medical University of Plovdiv
Comments to the Author:
The title, abstract and keywords accurately reflect the content of the manuscript and underlying data. The work includes 29 references. My recommendation is to cite more research articles from the last 2 years.
Sikonja, J.; Groselj, U.; Scarpa, M.; la Marca, G.; Cheillan, D.; Kölker, S.; Zetterström, R.H.; Kožich, V.; Le Cam, Y.; Gumus, G.; et al. Towards Achieving Equity and Innovation in Newborn Screening across Europe. Int. J. Neonatal Screen. 2022, 8, 31.
Koracin, V.; Mlinaric, M.; Baric, I.; Brincat, I.; Djordjevic, M.; Torkar, A.D.; Fumic, K.; Kocova, M.; Milenkovic, T.; Moldovanu, F.; et al. Current Status of Newborn Screening in Southeastern Europe. Front. Pediatr. 2021, 9, 648939.
Spiekerkoetter, U.; Bick, D.; Scott, R.; Hopkins, H.; Krones, T.; Gross, E.S.; Bonham, J.R. Genomic newborn screening: Are we entering a new era of screening? J. Inherit. Metab. Dis. 2023, 46, 778–795.
Iskrov, G.; Angelova, V.; Bochev, B.; Valchinova, V.; Gencheva, T.; Dzhuleva, D.; Dichev, J.; Nedkova, T.; Palkova, M.; Tyutyukova, A.; et al. Prospects for Expansion of Universal Newborn Screening in Bulgaria: A Survey among Medical Professionals. Int. J. Neonatal Screen. 2023, 9, 57.
And still others...
The author must systematically present and discuss the results of the research tasks and make detailed discussion and conclusion parts.
The author must summarize the conclusion and correspond to the tasks of the topic.
We thank Dr Hristova-Atanasova for their suggestion of additional references. We have amended the reference list to include these and another five primary studies in addition to the reviews we originally referenced.

Reviewer: 2
Dr. Lennart Hammarström , Karolinska Institute
Comments to the Author:
A straight forward protocol for genomic screening of 1000 newborn babies and considering "Australia" specific aspects of this type of studies. There are some additional studies already ongoing/published which might be added to the publication list, allowing comparison.
We thank Dr Hammarström for their comments and have expanded our reference list to include nine additional studies.

Reviewer: 3
Dr. Lianshu Han , Shanghai Jiao Tong University School of Medicine
Comments to the Author:
Authors in "Prospective cohort study of genomic newborn screening: BabyScreen+ study protocol" provided a three-phase process (pre-implementation, implementation and post-implementation

phases) for the evaluation of the feasibility of gNBS in Australia. The pre- and post-test processes were designed in great detail: the application of the online recruitment tools will facilitate education and consent of participants; multi-stage surveys and interviews were beneficial in assessing the acceptance and opinions of parents, HCP, and the Australian public about the program. However, the design of the implementation phase was not comprehensive enough, particularly in terms of technology assessment.
We thank Dr Han for their positive and constructive commentary on our paper.

1.As the authors mentioned (see "Study timetable and sites"), the pre-implementation phase of this project has been underway for a year. The details about the preparation of the materials (Page 9, lines 52-58) were suggested to be presented in the supplementary materials rather than stated in outline form. Screened conditions and selected genes included in the study should be listed in the supplementary materials.
The development of several of these resources will be subject to separate forthcoming publication. We have added the gene list as a supplementary item (Supplementary Table 1). The parental surveys are composed of standard instruments, which are listed in Table 1.

2.The interval between the birth of the child and the receipt of the test results should be as short as possible to ensure that results are returned within a clinically meaningful timeframe. Current research has been able to reduce this time interval to less than 20 days (PMID: 37656460). The time lag between enrollment and results in this program is up to 1.5 months (enrollment: 2 weeks after birth + results return: 4 weeks after sample collection), which is detrimental to the timely diagnosis and treatment of diseases (especially some genetic metabolic diseases).
While the reviewer is correct that there could be up to six weeks between birth and return of results, this is only expected to occur in exceptional circumstances. All participants will be recruited to the study during pregnancy and encouraged to complete enrolment before birth. We have amended the manuscript to add more detail on this, including information about follow-up for incomplete enrolment by the study team. New text is in bold and italicised.

This timeframe allows for exceptional circumstances, e.g., premature delivery, where enrolment is not completed before birth. However, in the majority of cases we expect enrolment to be completed during pregnancy. The study team will follow up incomplete enrolments two weeks before the expected due date *to ensure that results can be returned in a clinically meaningful timeframe.*

The four-week timeframe for sample testing is a maximum timeframe, comprised of two distinct blocks of up to two weeks. Block one is the maximum time expected to be taken by the standard NBS laboratory to complete their work. Cards will only be released once stdNBS is complete. The second block of two weeks is the time to process and analyse the genomic data. Time starts with the handover of the Guthrie card. Once fully implemented in practice, this would be expected to be faster as testing could be done in parallel with stdNBS. We have added more detail to the manuscript to clarify this.

StdNBS is expected to take up to two weeks. In order to comply with local requirements for access to NBS cards and avoid interference with stdNBS, BabyScreen+ will only have access to the sample once the routine process is complete.

The target timeframe for return of results following handover of the punches from the stdNBS laboratory is two weeks. Considering the two blocks of two weeks, *results will be returned within four weeks of sample collection.*

3.Does the participants pay for the gNBS? This may be an important factor in parental acceptance of the program acceptability of parents and the public.
gNBS is provided to participants free as part of this research study. We have clarified this in the methods:

gNBS and all required pre- and post-test support will be offered at no cost to birth parents.

4.Methods and analytical processes regarding sequencing need to be described in more detail, and references for analytical methods need to be cited rather than just the manufacturer.

The methods section has been updated with additional detail. Analysis exclusively uses the Illumina supplied toolkits, no other third-party components that could be cited are used. It is noted that analysis configurations are custom in-house design, which are validated and accredited to clinical standards.
*DNA will be extracted using **the Omega Biotek Mag-Bind DNA Blood and Tissue kit**. Following DNA extraction, PCR-free genome sequencing libraries will be created using the PCR-free DNA prep kit (Illumina) and sequenced **using a 2x150 base paired end read configuration** to an average depth of 30x on NovaSeq 6000 of X Plus instruments (Illumina).*

5.The value of gNBS is not fully exploited. Only variants that are classified as likely pathogenic or pathogenic will be reported and other information (carrier status, adult-onset conditions, or variants of uncertain significance) will be ignored, which will greatly affect the sensitivity of the test and result in compromised costs and benefits. It is recommended that a long-term follow-up process be added so that sequencing data can be reanalyzed when subjects develop disease phenotypes. The clinical significance of variants of uncertain significance (VUSs) in genes related to the existing phenotype and genes with moderate or limited evidence of causing the specific indication, will be re-evaluated based on indications, further segregation analysis and clinical evaluation. Furthermore, carrier-status information is helpful in future reproductive planning at a time when families are having children.
We thank the reviewer for their suggestions. However, this study is a pilot, focussed on assessing the feasibility of delivering a genomic newborn screening program in one Australian state. As such, the suggestions presented here, while of great interest to the authors, are outside the scope of this research project. We hope to extract additional value from the genomic data in future studies, subject to additional funding.

As this is clinically accredited data, it will be available for future healthcare use as participants develop disease phenotypes. We plan to collect data on reuse for the duration of the study (5 years) and have added this to the manuscript.

Clinical and laboratory data from RCH and VCGS will be accessed and examined to establish if and for what purpose genomic data generated at birth has been accessed and/or re-analysed within five years post-result.

However, we acknowledge this will not fully explore the use of the genomic data, and have added this to the Strengths and Limitations:

- ***As this is a pilot study, we are unable to fully assess the value of stored genomic data for lifelong healthcare use.***

6.What does stdNBS refer to? Is it tandem mass spectrometry screening?
We have made sure the first instance of standard NBS (stdNBS) includes the full term in the introduction and have provided a link to a list of conditions currently include in Australia.

7.The authors mentioned in the “secondary aims” to compare the performance of stdNBS and gNBS, which is critical for evaluating the diagnostic performance and clinical utility of gNBS. Authors need to describe the comparison process and indicators in detail.
In addition to measuring the concordance of stdNBS and gNBS results we have added detail to the evaluation section on how these will be further compared.

Performance of gNBS will be further evaluated against stdNBS where a genetic diagnosis is confirmed. We will collect data on subsequent clinical management, including the timing of commencement of therapies and downstream healthcare utilisation. Where possible, we will compare this against historical controls from VCGS and the Royal Children’s Hospital (patients diagnosed with the same disorder in the last 10 years). Cost-effectiveness and cost-benefit analyses will be conducted to evaluate whether the additional cost of gNBS relative to stdNBS is outweighed by longer-term cost-savings and improvements in diagnostic, clinical, and personal outcomes for children and families.

Minor issue:

"stdNBS" and REDCap should be labeled with their full names at the time of initial appearance.
Thank you for alerting us to this. We have spelled out both names the first time they appear in the manuscript.

VERSION 2 – REVIEW

REVIEWER	Hristova-Atanasova, Eleonora Medical University of Plovdiv, Social medicine and Public Health
REVIEW RETURNED	14-Dec-2023

GENERAL COMMENTS	I express my gratitude for your responses. The manuscript has been substantially revised, and the authors have furnished precise and detailed explanations. I advised the acceptance of the manuscript.