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TABLE OF CONTENTS

[Intervention Protocol]

Next-generation sequencing (NGS) techniques for pre-symptomatic identification of genetic diseases in newborns

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ABSTRACT

Objectives

This is a protocol for a Cochrane Review (intervention). The objectives are as follows:

To evaluate the benefits and harms of using NGS techniques compared to conventional newborn screening alone for pre-symptomatic identification of genetic diseases in newborns.

Secondary objectives: to explore equity and ethical issues in the application of the new techniques, to inform healthcare decisions by families, carers, and policymakers.

BACKGROUND

Description of the condition

Genetic diseases are disorders resulting from changes (mutations) in an individual's deoxyribonucleic acid (DNA) [1]. DNA carries genetic information that makes each of us who we are, such as the color of our hair and the chance of having health conditions. Mutations may occur in a single gene (monogenic disorders), in two or more genes (polygenic disorders), or may be multifactorial: that is, polygenic disorders impacted by environmental factors [2, 3]. Polygenic and multifactorial conditions are caused by the combined action of more than one gene, lifestyle and environmental factors, do not have a clear pattern of inheritance, can have a heterogeneous presentation even within the same family, and cannot definitely be identified by a genetic test. Examples of monogenic disorders include cystic fibrosis, phenylketonuria (PKU; Supplementary material 2), and spinal muscular atrophy. Examples of polygenic/multifactorial conditions are hypertension, refractive errors, and diabetes.

Genetic diseases could also be classified in relation to the chance that a change in the DNA sequence results in an altered clinical phenotype. This is known as gene penetrance. A high penetrance genetic mutation is one that almost always results in the individual expressing the associated disorder or trait if they carry the mutation. This means that the likelihood of developing the condition when the mutation is present is very high. High-penetrance monogenic diseases detectable through next-generation sequencing (NGS) used as part of newborn screening are the major focus of our review.

Newborn screening is an important preventive strategy with consequences for public health [4]. Its goal is to achieve pre-symptomatic identification of treatable diseases for which timely intervention can provide significant future clinical benefits. It consists of pricking the heel of a newborn to collect a small amount of blood, which is then analyzed for specific metabolites that may be altered even before the clinical presentation of a congenital disease. Newborn screening programs began in the 1960s with tests for phenylketonuria (PKU), using the bacterial inhibition assay technique [5]. Before the introduction of the test, PKU was a common cause of intellectual disability, but is now treated using dietary management, and affected children achieve normal developmental outcomes [5]. Tandem mass spectrometry (MS/MS), an important innovation of the 1980s, made it possible to test for more than 40 metabolic disorders [6]. Conditions identified using MS/MS include inborn errors of metabolism (IEMs), such as organic acidurias and fatty acid oxidation deficits. Some unfavorable outcomes related to these conditions can be prevented or reduced by early diagnosis through newborn screening [7, 8]. Newborn screening programs are employed worldwide, and although the number and type of tests differ, newborn screening is recognized as a vital public health intervention that reduces negative health consequences and mortality [6, 9, 10, 11].

The choice of the conditions to be included in newborn screening panels is crucial and should follow Wilson and Jungner's principles [12]. Thus, we expect to find a screening panel including epidemiologically relevant diseases, with an impact on public health due to their prevalence, their consequences for the lives of patients, and their costs to society. Other important characteristics for a condition to be included in a screening program are to have a recognizable latent or early symptomatic stage, together with the availability of treatments that can modify the clinical course of the disease. These features are well represented in the case of congenital hypothyroidism. It occurs in one in 3000 to 4000 infants [13]. Newborns are asymptomatic during the first days of life, and replacement therapy with levothyroxine, if started early after birth, can prevent the severe neurocognitive delay caused by a late diagnosis [14].

The advancement of screening techniques has prompted a revision of the classic screening principles [15]. Policymakers have expanded newborn screening programs to include conditions that may not have immediate treatments, but for which early diagnosis can significantly alter disease management and family planning decisions.

Description of the intervention and how it might work

Even though newborn screening has greatly evolved and improved since its introduction in clinical practice, it still carries important limitations. First, there is the need for identifiable biochemical markers whose blood levels can suggest a specific disease. Unfortunately, there are clinically relevant disorders that lack reliable single biochemical markers for early detection, such as proximal urea cycle disorders [16]. Second, newborn screening tests cannot be performed until at least 24 hours after birth because some metabolites need time to reach a threshold level; this is particularly important in preterm infants because their metabolism is immature. Preterm infants usually require multiple repeats of newborn screening in order to catch all the biochemical changes that could be missed in the first days of life.

The answer to some of these limitations could be provided by new genetic techniques such as next-generation sequencing (NGS). Genetic tests could also potentially identify a greater number of conditions, as about 80% of rare diseases – globally accounting for nearly 300 million people worldwide – are known to be of genetic origin [17, 18].

Next-generation sequencing (NGS) is a newer technology and is not standard in many newborn screening programs. NGS includes whole genome sequencing (WGS), whole exome sequencing (WES), and rapid whole genome sequencing (rWGS), introduced in 2005, 2009, and 2012, respectively [19, 20, 21]. NGS, also called “massively parallel” sequencing, enables data sequencing from thousands of DNA templates simultaneously. Customized panels including a limited number of genes can be created. NGS reduces time and costs compared with traditional methods such as Sanger sequencing [22]. For example, while Sanger sequences one DNA fragment at a time, NGS can sequence millions of fragments simultaneously. NGS testing may be especially useful for disorders lacking a reliable single biochemical marker and can be performed soon after birth.

Given the potential of NGS to identify more diseases in a cost-effective and timely manner, it is important to understand if newborn screening with NGS (compared to newborn screening without NGS) improves early diagnosis and leads to changes in clinical management, and whether these changes, in turn, improve outcomes, and reduce morbidity and mortality in infants with pre-symptomatic diseases. Even if current costs are lower than in the past, NGS could still be too expensive. Further, some techniques might be unavailable in some countries, thus raising

equity-related concerns. Moreover, the benefits of identifying more conditions must be balanced with ethical, medical, economic, and social considerations. One such consideration is overscreening, defined as "the excessive or unnecessary use of screening tests or procedures for detecting diseases or conditions in individuals who are not at high risk or who are unlikely to benefit from such screenings" [23]. The negative effects of overscreening may include psychological distress, false-negative or false-positive results, and overtreatment [23]. It is, therefore, important for clinicians to understand when and to what extent NGS should be used in newborn screening and to consider the true impact of incidental and uncertain findings on patients and families in clinical practice. We should also be aware that some genetic mutations could have a different prevalence in different ethnic groups, requiring specific adjustments for the interpretation of the results. Other issues regarding genetic screening that should be considered concern informed consent, data privacy, and return of results. The American College of Medical Genetics and Genomics (ACMG) has given healthcare providers a set of guidelines on these issues for now, and plans to keep updating this guidance as NGS techniques and debates about ethical responsibilities evolve [24; 25].

Why it is important to do this review

Newborn screening stands as a crucial preventive measure with significant implications for public health. Recent advancements in genetic techniques such as NGS offer the opportunity to expand screening to a greater number of diseases. The potential of these new techniques has attracted considerable interest, and led to the initiation of large-scale studies and initiatives, including the European Screen4Care consortium and the Newborn Programme in the UK [1, 26, 27, 28]. A careful and reasoned application of these new technologies to augment traditional screening strategies could allow greater and earlier access to treatment and prevention strategies with positive outcomes for children, families, and society. Risks should also be taken into account. This review aims to provide a rigorous and comprehensive synthesis of the currently available evidence, as a global assessment of this evidence could help inform future application of NGS techniques.

OBJECTIVES

To evaluate the benefits and harms of using NGS techniques compared to conventional newborn screening alone for pre-symptomatic identification of genetic diseases in newborns.

Secondary objectives: to explore equity and ethical issues in the application of the new techniques, to inform healthcare decisions by families, carers, and policymakers.

METHODS

For this protocol, we have followed methodological guidance in the *Cochrane Handbook for Systematic Reviews of Interventions* and the Preferred Reporting Items for Systematic review and Meta-Analysis Protocols (PRISMA-P) reporting guidance [29, 30]. For the review, we will follow methodological guidance in the *Cochrane Handbook for Systematic Reviews of Interventions* [29] and Cochrane's MECIR (Methodological Expectations for Cochrane Intervention Reviews) guidelines [31], and report the review according to PRISMA guidelines [32, 33].

Criteria for considering studies for this review

Types of studies

We will include randomized controlled trials (RCTs) and quasi-RCTs. We define quasi-RCTs as RCTs where allocation is decided by a process which is not completely random and unpredictable, such as allocation by patient identification (ID) number. We will include cross-over studies (a type of clinical trial in which all participants receive the same two or more interventions), but will only include data from the first phase of the study (i.e. before participants receive the second intervention).

We will exclude cluster-RCTs, where participants are randomized as groups and not as individuals, because we are only interested in studies where the unit of analysis is the single participant.

We plan to include studies reported as full-text and those published as abstracts. We will include studies published in any language. We will include records of ongoing trials meeting our inclusion criteria in an ongoing studies reference list.

Types of participants

We will include two populations of newborns:

- infants undergoing initial newborn screening: term and preterm infants, within the first week of life;
- infants undergoing retesting, following uncertain or slightly positive results (altered but not reaching the diagnostic threshold) that need confirmation:
  - term infants of less than 28 days of age; or
  - preterm infants of less than 44 weeks' corrected gestational age.

Preterm infants are defined as born before 37 weeks of gestational age (born before expected due date). We decided to keep these two populations separate as some conditions included in the screening protocols can have a different incidence in term and preterm infants. More importantly, local screening strategies usually differ in these populations in order to reduce false-positive or false-negative results linked to the different levels of maturation of these newborn groups [34, 35].

We will include newborns screened in a hospital setting both as inpatients or outpatients.

We will include studies enrolling both newborns (< 28 days of life for term infants or < 44 weeks of corrected gestational age for preterm infants) and older infants by contacting study authors to obtain separate outcome data for the newborns. If we are unsuccessful in obtaining separate data for newborns, we will exclude studies where the mean age of the included participants exceeds 28 days in term infants and 44 weeks of corrected gestational age in preterm infants.

We will exclude studies if an ethics committee has not approved their protocol.

Types of interventions

We will include studies of initial or retest newborn screening, with or without NGS techniques, to identify monogenic conditions caused by a single gene and with high penetrance.

We define 'initial screening' as that undertaken within the first week of life, and 'retest screening' as testing following an initial newborn screening that did not provide definitive or certain results (either positive or negative). Retest screening may be undertaken at any time after the initial screening, and may be delivered more than once. Initial screening and retesting may be conducted on either inpatients or outpatients.

The interventions of interest are the following:

- NGS techniques for initial screening, including, but not limited to, targeted gene panels, whole genome sequencing, or whole exome sequencing;
- NGS techniques for retests, including, but not limited to, targeted gene panels.

The review's comparison of interest is newborn screening with NGS versus newborn screening alone.

Interventions are delivered once on the same infant during the newborn screening process, either as initial newborn screening or as retest.

Outcome measures

The following outcome measures do not form part of the eligibility criteria.

Critical outcomes

For the following outcomes, we consider the first hospital admission to be the first hospitalization other than the maternity ward hospital stay.

- Changes in clinical management, defined as changes in therapeutic interventions or hospital plan. This outcome will be evaluated during the first hospital admission or, in an outpatient setting, during the first visit following screening results.
- Actionability of results, expressed using ClinGen actionability semiquantitative metric (CASQM) [36]
- Morbidity due to late or no diagnosis of a condition which could have been treated. Any pathologic condition or adverse event resulting from late or no diagnosis of a genetic disease which could have been treated if recognized in a timely fashion by newborn screening. We will consider two time points: during the first hospital admission and on subsequent visits.
- Death as a result of late or no diagnosis of a condition that could have been treated. We will consider two time points: during the first hospital admission and on subsequent visits.
- Adverse events: measured only once, after receiving test results, as follows:
  - incidental findings, defined as unwanted diagnoses (usually untreatable or irrelevant diseases or conditions with an impact later in life and not during childhood);
  - uncertain findings, defined as results that do not have a clear clinical significance.

Important outcomes

The following outcomes will be measured during first hospital admission and on subsequent visits.

- Results with consequences for the family (new diagnosis in parents or other relatives)
- Parental distress (different domains)
  - Parent-child relationship, assessed with Mother-to-Infant Bonding Scale [37], Parent-Child Relationship Scale (P-CRS) [38], or other instrument. If more than one measure is available in each study, we will prioritize the Mother-to-Infant Bonding Scale because it is validated in the first years of life.
  - Parents' relationship, assessed with Kansas Marital Satisfaction Scale [39], Perceived Quality of Marital Relationship Scale [40], Satisfaction with Married Life Scale (SWML) [41], or other instrument. If more than one measure is available in each study, we will prioritize the Kansas Marital Satisfaction Scale because it is validated and more widely used.
  - Parents' psychological distress, assessed with a validated scale (e.g. Edinburgh Postnatal Depression Scale anxiety subscale [42, 43]; the 6-item Kessler psychological distress scale instrument [44]; Depression, Anxiety, Stress Scale-21 [DASS-21]). If more than one measure is available in each study, we will prioritize the Depression, Anxiety, Stress Scale-21 because it is not specific to post-natal depression (unlike the Edinburgh Postnatal Depression Scale), and it has been studied in and applied to different ethnicities.

The following outcomes will be measured only once, after receiving test results.

- Denied consent (number of families that refused newborn screening).
- Time to final test results (when a conclusive result is reached, either positive or negative).
- Additional testing requested after receiving test results.

Search methods for identification of studies

Electronic searches

An Information Specialist (MF) has written a draft strategy for MEDLINE (Ovid), which will be translated using controlled vocabulary and syntax for other databases (Supplementary material 1). We will limit search results using methodological filters to identify randomized clinical trials and systematic reviews. We will state the source of the filters in the search strategies. An Information Specialist will peer-review the MEDLINE search strategy. Searches will be conducted without language, publication type, or publication status restrictions. Searches for trials will be run without date limits; searches for systematic reviews will be limited to the past two years. We will search the following databases:

- Cochrane Central Register of Controlled Trials (CENTRAL) in the Cochrane Library;
- Ovid MEDLINE All (1946 to the present);
- Ovid Embase (1974 to the present);
- Epistemonikos (for systematic reviews).

We will search two clinical trials registries for ongoing or recently completed trials:

- ClinicalTrials.gov, within the US National Library of Medicine;
- the World Health Organization's International Clinical Trials Registry Platform.

We will report search and study selection details in a PRISMA flow diagram [32].

Searching other resources

We will search the following conference abstracts to the most current year available:

- European Academy of Paediatric Societies (EAPS), from 2020;
- Pediatric Academic Societies (PAS), from 2018;
- Perinatal Society of Australia and New Zealand (PSANZ), from 2014.

We will review the reference lists of included studies and related systematic reviews. We will search for errata or retractions for included studies in PubMed (www.ncbi.nlm.nih.gov/pubmed) and Retraction Watch (retractiondatabase.org). We will contact study authors should we have questions regarding data reported in trials selected for inclusion.

Data collection and analysis

We will collect information regarding the method of randomization, blinding, intervention(s), and whether the trial was single center or multicenter for each included study. We will note information regarding trial settings and participants, including gestational age, postnatal age, weight, and transfusions received. We will analyze the clinical outcomes noted in the Outcome measures. Where studies have multiple publications, we will collate the reports of the same study, so that each study, rather than each report, is the unit of interest for the review, and such studies have a single identifier with multiple references. In the event we identify and include studies by review authors, we will have two independent review authors undertake the following: screening and selection; data extraction; risk of bias assessment; and assessment of certainty of evidence. In the event multiple review authors are involved in an included study, we will recruit independent colleagues to undertake these tasks.

Selection of studies

We will download all titles and abstracts retrieved by electronic searches to a reference management software and remove duplicates. We will conduct screening using Covidence [45]. If the searches identify more than 2000 references, we may use two components of Cochrane's Screen4Me (S4M) tool (https://training.cochrane.org/online-learning/good-practice-resources-cochrane-authors/screen4me), Known Assessments and RCT Classifier, to reduce screening activities by the review authors. S4M includes a third utility, Cochrane Crowd. A decision to use Cochrane Crowd will be contingent on total retrieval and the timeline proposed for classification by the Crowd. Information about S4M may be found in the literature [46, 47, 48, 49]. We will document results of S4M assessment, if used, in the review.

Two review authors (SP, MBo) will independently screen titles/abstracts; they will then independently review the full-texts of references retained following title/abstract review. At any point during the screening process, we will resolve disagreements by discussion or by consulting a third review author (MBr or FF). We will document the reasons for excluding studies during full-text review in a 'Characteristics of excluded studies' table. We will provide any information we can obtain about ongoing studies. We will present studies with insufficient information to allow data extraction in an 'awaiting assessment' reference list, and they will be incorporated into an update of the review should sufficient information become available. We will group multiple reports of the same study under a single study ID so that each study, rather than each report, will be the unit of interest in the review. We will record the study selection process in sufficient detail to complete a PRISMA flow diagram [32].

Data extraction and management

We will extract study data using a form based on the Cochrane Effective Practice and Organization of Care Group data collection checklist [50]. We will pilot the form within the review team using a sample of three included studies. Two review authors (SP, MBo) will independently extract data for the trials that meet the inclusion criteria; data from each review author will be compared and disagreements resolved by discussion.

We will extract the following characteristics from each included study.

- Administrative details: study author(s); published or unpublished; year of publication; year in which study was conducted; presence of vested interests of the study authors.
- Study characteristics: study registration, study design type, study setting, number of study centers and location; informed consent; ethics approval, completeness of follow-up (e.g. greater than 80%).
- Participants: number randomized, number lost to follow-up/withdrawn, number analyzed, mean gestational age, gestational age range, mean chronological age, chronological age range, sex, birth weight, weight at the screening, inclusion criteria and exclusion criteria.
- Interventions: type of intervention, used as an add-on strategy or alone.
- Outcomes as described above (in Outcome measures).

In order to complete our planned equity-related assessment, we will also extract the following PROGRESS-Plus characteristics:

- place of residence (country);
- ethnicity/culture;
- education;
- socioeconomic status.

We will describe ongoing studies identified by our search and document available information (including the primary author, research question(s), methods, and outcome measures, together with an estimate of the anticipated reporting date) in the 'Characteristics of ongoing studies' table. Should any queries arise, or in cases for which additional data are required, we will contact study investigators/authors for clarification. Two review authors (SP, MBo) will use Review Manager (RevMan) for data entry [51].

Risk of bias assessment in included studies

Two review authors (SP, MBo) will independently assess risk of bias. We will resolve disagreements by discussion or by consulting a third review author (KJJ or FF). We will undertake risk of bias assessment according to Chapter 7 and Chapter 8 of the *Cochrane Handbook for Systematic Reviews of Interventions* [52, 53]. We will use the RoB 2 tool to assess bias for intention-to-treat (ITT) effects for the critical outcomes and adverse effects. We will assess the outcomes listed in Certainty of the evidence assessment.

We will assess items under the following domains:

- risk of bias in the randomization process;
- bias due to deviations from intended interventions;
- bias due to missing outcome data;
- bias in measurement of the outcome;
- bias in selection of the reported result.

We will use the RoB 2 Excel tool to assess individually randomized, parallel-group trials. Using the signaling questions in the RoB 2 tool, we will rate each domain as having low risk, some concerns, or high risk of bias. We will summarize risk of bias judgments across studies for each of the domains listed for each outcome. A judgment of high risk of bias within any domain has the same implications for the result overall, regardless of which domain is assessed as high risk. Therefore, if the answers to the signaling questions yield a judgment of high risk of bias, we will consider whether any identified problems are of sufficient concern to warrant a high-risk judgment for the result overall. When we judge that there are 'some concerns' for bias in multiple domains, we will consider an overall judgment of high risk of bias for that result or group of results. We will consider the overall RoB 2 judgment for each outcome as part of the GRADE assessment presented in our summary of findings table.

We plan to perform a sensitivity analysis comparing studies at high overall risk of bias to those at low overall risk of bias. We will use the results of the analysis to explore or explain possible causes of heterogeneity between studies.

Measures of treatment effect

For dichotomous data, we will present results using risk ratios (RR) and risk differences (RD) with 95% confidence intervals (CIs). We will calculate the number needed to treat for an additional beneficial outcome (NNTB), or number needed to treat for an additional harmful outcome (NNTH) with 95% CIs if there is a statistically significant reduction (or increase) in RD.

For continuous data, we will use the mean difference (MD) when outcomes were measured in the same way between trials. We will use the standardized mean difference (SMD) to combine trials that measured the same outcome but used different methods. If trials report continuous data as a median and interquartile range (IQR) and data pass the test of skewness, we will convert the median to a mean and estimate the standard deviation as IQR/1.35.

If trials report data in an unusable format for entering directly into a meta-analysis, we will convert the data to the required format, using the information in Chapter 6 of the *Cochrane Handbook for Systematic Reviews of Interventions* [54].

Unit of analysis issues

We will perform the primary analysis per individual randomized.

If trials have multiple arms that are compared to the same control condition that will be included in the same meta-analysis, we will either combine groups to create a single pair-wise comparison, or select one pair of interventions and exclude the others.

In the meta-analysis and data synthesis, we will only include the first-phase data from cross-over trials.

Dealing with missing data

We intend to carry out analysis on an intention-to-treat basis for all included outcomes. Whenever possible, we will analyze all participants in the treatment group to which they were randomized, regardless of the actual treatment received. If we identify important missing data (in the outcomes) or unclear data which cannot be derived, we will approach the analysis as follows.

- We will contact the original study investigators to request the missing data. Where possible, we will impute missing standard deviations (SDs) using the coefficient of variation (CV) or calculate SDs from other available statistics, including standard errors, confidence intervals, t values, and P values.
- We will make explicit the assumptions of any methods used to deal with missing data.
- If the data are assumed to be missing at random, we will analyze the data without imputing any missing values. If this cannot be assumed, then we will impute the missing outcomes with replacement values, assuming all participants have had a poor outcome, and we will conduct sensitivity analysis to assess any changes in the direction or magnitude of effect resulting from data imputation.

We will address the potential impact of missing data on the findings of the review in the 'Discussion' section.

Reporting bias assessment

We will assess reporting bias by comparing the stated critical and important outcomes with reported outcomes. Where study protocols are available, we will compare these to the full publications to determine the likelihood of reporting bias. We will document studies using the interventions in a potentially eligible infant population but not reporting on any of the primary and secondary outcomes in the 'Characteristics of included studies' tables.

We will use funnel plots to screen for publication bias where there are a sufficient number of studies (> 10) reporting the same outcome. If publication bias is suggested by a significant asymmetry of the funnel plot on visual assessment, we will incorporate this in our assessment of the certainty of the evidence [55]. If our review includes fewer than 10 studies eligible for meta-analysis, the ability to detect publication bias will be largely diminished, and we will simply note our inability to rule out possible publication bias or small-study effects.

Synthesis methods

If we identify multiple studies that we consider to be sufficiently similar, we will perform meta-analysis using Review Manager [51]. For categorical outcomes, we will calculate the typical estimates of RR and RD, each with its 95% CI; for continuous outcomes, we will calculate the MD or the SMD, each with its 95% CI. We will use a random-effects model to combine data. If there is evidence of clinical heterogeneity (see Investigation of heterogeneity and subgroup analysis), we will try to explain this based on the different study characteristics, sensitivity analysis, and the planned subgroup analyses. We will create forest plots to provide a graphical representation of the study data.

If we judge meta-analysis to be inappropriate, we will refer to methodological guidance from Chapter 12 of the *Cochrane*

Handbook for Systematic Reviews of Interventions [56], and synthesis without meta-analysis (SWiM) reporting guidance [57]. We will create a table with studies ordered by risk of bias, and calculate standardized effect estimates for each study. This table will be modeled on the worked example, Table 12.4.b, from the *Cochrane Handbook for Systematic Reviews of Interventions* [56]. We will create a forest plot to provide a graphical representation of the data.

The primary analysis will include all eligible studies; sensitivity analysis on studies at overall high and low risk of bias will be performed.

Investigation of heterogeneity and subgroup analysis

We will describe the clinical diversity and methodological variability of the evidence narratively and in tables. Tables will include data on study characteristics, such as design features, population characteristics, and intervention details.

To assess statistical heterogeneity, we will visually inspect forest plots and describe the direction and magnitude of effects and the degree of overlap between confidence intervals. We will also consider the statistics generated in forest plots that measure statistical heterogeneity. We will use the I^2 statistic to quantify inconsistency between the trials in each analysis. We will also consider the P value from the Chi^2 test to assess if this heterogeneity is significant ($P < 0.1$). If we identify considerable heterogeneity [58], we will report the finding and explore possible explanatory factors, looking at study characteristics, using prespecified subgroup analysis and sensitivity analyses.

We will grade the degree of heterogeneity as:

- 0% to 40% might not represent important heterogeneity;
- 30% to 60% may represent moderate heterogeneity;
- 50% to 90% may represent substantial heterogeneity;
- more than 75% may represent considerable heterogeneity.

We will use a rough guideline to interpret the I^2 value rather than a simple threshold, and our interpretation will take into account the understanding that measures of heterogeneity (I^2 and Tau) will be estimated with high uncertainty when the number of studies is small [58].

We will interpret tests for subgroup differences in effects with caution, given the potential for confounding with other study characteristics and the observational nature of the comparisons. In particular, subgroup analyses with fewer than five studies per category are unlikely to be adequate to ascertain valid differences in effects and will not be highlighted in our results. When subgroup comparisons are possible, we will conduct stratified meta-analysis and a formal statistical test for interaction to examine subgroup differences that could account for effect heterogeneity (e.g. Cochran's Q test, meta-regression) [58, 59].

Given the potential differences in intervention effectiveness related to prematurity, as discussed in the Background, we will conduct subgroup comparisons to see if the intervention is more effective for term or preterm infant screening. We will only perform subgroup analysis on prespecified outcomes where we think that the subgroup could have an impact. We plan to carry out the following subgroup analyses of factors that may contribute to heterogeneity in the effects of the intervention, and we will use the following outcomes in subgroup analyses if there are enough studies reporting to support valid subgroup comparisons (at least five studies per subgroup):

Preterm versus term infants for the following outcomes

- Changes in clinical management
- Morbidity due to late or no diagnosis of a condition which could have been treated
- Death as a result of late or no diagnosis of a condition which could have been treated
- Actionability of results
- Time to final test results
- Adverse events:
  - incidental findings
  - uncertain findings

Low-income countries versus middle- and high-income countries for the following outcomes

- Changes in clinical management
- Morbidity due to late or no diagnosis of a condition which could have been treated
- Death as a result of late or no diagnosis of a condition which could have been treated
- Actionability of results
- Denied consent

Ethnicity for the following outcomes

- Morbidity due to late or no diagnosis of a condition that could have been treated
- Death as a result of late or no diagnosis of a condition that could have been treated
- Parental distress (different domains)
  - Parent-child relationship, evaluated through different scales (e.g. Mother-to-Infant Bonding Scale and Parent-Child Relationship Scale [P-CRS])
  - Parents' relationship, evaluated through different scales (e.g. Kansas Marital Satisfaction Scale; Perceived Quality of Marital Relationship Scale; Satisfaction With Married Life [SWML] scale)
  - Parents' psychological distress, evaluated through different validated scales (e.g. Edinburgh Postnatal Depression Scale anxiety subscale; 6-item Kessler psychological distress scale; Depression, Anxiety, Stress Scale-21 [DASS-21])
- Denied consent

Healthy babies versus ill-appearing babies

- Changes in clinical management
- Morbidity due to late or no diagnosis of a condition which could have been treated
- Death as a result of late or no diagnosis of a condition which could have been treated
- Actionability of results
- Time to final test results
- Adverse events:
  - incidental findings
  - uncertain findings

- Denied consent

Disorder type (biochemical disorders, immune deficiencies, neurodevelopmental diseases, other genetic diseases/ syndromes without biochemical markers)

- Changes in clinical management
- Morbidity due to late or no diagnosis of a condition which could have been treated
- Death as a result of late or no diagnosis of a condition which could have been treated
- Actionability of results
- Time to final test results

We will use a random-effects model to analyze data from different subgroups.

Equity-related assessment

We will report PROGRESS-Plus (place of residence, race/ethnicity/ culture/language, occupation, gender/sex, religion, education, socioeconomic status, social capital, age, sexual orientation, and disability) characteristics found in the studies included in our review [60]. We will aim to determine if any of the characteristics influenced the equitable use or uptake of NGS screening methods. We will report the PROGRESS-Plus data in a table and summarize any findings in our discussion.

We will conduct subgroup analyses on prespecified outcomes based on country income status (i.e. low-income, lower middle-income, upper middle-income, high-income; based on World Bank categorizations) and ethnicity as reported in the studies, in an effort to ascertain if either influence the applicability of NGS.

Sensitivity analysis

We plan to perform a sensitivity analysis restricted to studies assessed as having a low risk of bias to explore the robustness of the main analysis.

If we need to impute missing outcomes with replacement values based on assuming all participants have had a poor outcome, we will conduct sensitivity analysis to assess any changes in the direction or magnitude of effect resulting from data imputation.

If we notice relevant differences between studies, we will perform a sensitivity analysis to explore whether the differences are due to the use of targeted gene panels versus more expanded analysis (i.e. WES, WGS), and conventional newborn screening with only a core panel of disorders (conventionally using the Recommended Uniform Screening Panel [61]) versus expanded conventional newborn screening.

Given that there is no formal statistical test that can be used for sensitivity analysis, we will make informal comparisons between the different ways of estimating the effect under different assumptions. Changes in the P values should not be used to judge whether there is a difference between the main analysis and sensitivity analysis, since statistical significance may be lost with fewer studies included. We will report sensitivity analysis results in tables rather than forest plots.

Certainty of the evidence assessment

We will use the GRADE approach, as outlined in the GRADE Handbook [62], to assess the certainty of evidence for the following (clinically relevant) outcomes in the comparison of conventional newborn screening versus conventional newborn screening plus NGS in initial screening. We will report results using the narrative statements recommended in the *Cochrane Handbook* and GRADE guidance [63, 64].

- Changes in clinical management, defined as changes in therapeutic interventions or hospital plan. Time point: during the first hospital admission.
- Morbidity due to late or no diagnosis of a condition which could have been treated. Any pathologic condition or adverse event resulting from late or no diagnosis of a genetic disease which could have been treated if recognized in a timely fashion by newborn screening. Time point: visits after first hospital admission.
- Death as a result of late or no diagnosis of a condition which could have been treated. Time point: visits after first hospital admission.
- Actionability of results expressed using ClinGen actionability semiquantitative metric (CASQM) [36]. Time point: during the first hospital admission.
- Adverse events:
  - incidental findings, defined as unwanted diagnoses (usually untreatable or irrelevant diseases or conditions with an impact later in life and not during childhood)
  - uncertain findings, defined as results that do not have a clear clinical significance

Two review authors (SP, MBo) will independently assess the certainty of the evidence for each of the outcomes above. We will resolve disagreements by discussion or by consulting a third review author (KJJ or FF). We will use the overall RoB 2 assessments to inform our GRADE judgments. We will consider evidence from randomized controlled trials as high certainty, downgrading the evidence one level for serious (or two levels for very serious) limitations based upon the following: design (risk of bias), consistency across studies, directness of the evidence, precision of estimates, and presence of publication bias.

The GRADE approach results in an assessment of a body of evidence at one of the following four grades:

- high certainty: we are very confident that the true effect lies close to that of the estimate of the effect;
- moderate certainty: we are moderately confident in the effect estimate: the true effect is likely to be close to the estimate of the effect, but there is a possibility that it is substantially different;
- low certainty: our confidence in the effect estimate is limited: the true effect may be substantially different from the estimate of the effect;
- very low certainty: we have very little confidence in the effect estimate: the true effect is likely to be substantially different from the estimate of effect.

We will justify all decisions to downgrade the certainty of the evidence using footnotes, and will make comments to aid the reader's understanding of the review where necessary. We will report results with reference to Cochrane's MECIR Manual,

standards for the reporting of new Cochrane intervention reviews [31]. We will report results in accordance with recommended narrative statements, as described in Chapter 15, Table 15.6.b of the *Cochrane Handbook for Systematic Reviews of Interventions* [63].

We will use GRADEpro software to create a summary of findings table to report the certainty of the evidence for the comparison between conventional newborn screening (according to local policies) versus conventional newborn screening (according to local policies) plus NGS in initial screening [65].

We will conduct the review according to this published protocol and report any deviations from it in the 'Limitations of the review process' section of the systematic review.

Consumer involvement

This protocol includes consumer peer review facilitated by the Cochrane Editorial Service. Using the Cochrane Engage platform, we will submit a short questionnaire to parents of infants on concerns about their child being exposed to NGS, and to physicians about their experience using these techniques. Their answers – reflecting consumers' points of view – will guide us in discussing the review results. We will also use consumers' contributions to help us in writing the plain language summary. These tasks will be available at Cochrane Engage (https://engage.cochrane.org/tasks/3513).

SUPPLEMENTARY MATERIALS

Supplementary materials are available with the online version of this article: 10.1002/14651858.CD016118.

Supplementary material 1 Search strategies

Supplementary material 2 Definitions

ADDITIONAL INFORMATION

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Editorial and peer-reviewer contributions

Cochrane Neonatal supported the authors in the development of this review.

The following people conducted the editorial process for this article:

- Sign-off Editor (final editorial decision): Mohan Pammi MD, PhD, Professor, Dept. of Pediatrics, Baylor College of Medicine.
- Managing Editor (selected peer reviewers, provided editorial guidance to authors, edited the article): Jenny Bellorini, Cochrane Central Editorial Service.
- Editorial Assistant (conducted editorial policy checks, collated peer-reviewer comments and supported the editorial team): Addie-Ann Smyth, Cochrane Central Editorial Service.
- Copy Editor (copy editing and production): Faith Armitage, Cochrane Central Production Service.
- Peer reviewers (provided comments and recommended an editorial decision): Stephen Kingsmore (clinical/content review); Hariklia Nguyen (consumer review); Jo-Ana Chase, Cochrane Evidence Production and Methods Directorate (methods review); Jo Platt, Central Editorial Information Specialist (search review). An additional peer reviewer provided clinical/content peer review but chose not to be publicly acknowledged.

Contributions of authors

MBr and SP oversaw the project and are responsible for updating the review.

Contributions of authors are/will be as follows:

- Conception of the review: RS, SP, and MBr
- Design of the review: SP, RS, and MBr
- Co-ordination of the review: SP with support from MBr and MF
- Literature search: MF
- Selection of studies for inclusion in the review: SP, MBo, and FF
- Collection of data for the review: SP, MBo, and FF
- Assessment of the risk of bias in the included studies: SP, MBo, and KJJ
- Analysis of data: SP, MBo, FF, KJJ, and MBr
- Assessment of the certainty in the body of evidence: SP, MBo, FF, KJJ and MBr
- Interpretation of data: SP, MBo, FF, KJJ, RS, and MBr
- Writing of the review: SP, MBo, FF, KJJ, MF, and MBr
- Final submission reviewed and approved by all authors

Declarations of interest

S Pessano has no interests to declare.

M Boldor has no interests to declare.

F Faravelli has no interests to declare.

M Fiander is a Managing Editor and Information Specialist for Cochrane Neonatal; however, she did not participate in the editorial process for this protocol.

KJ Jørgensen has no interests to declare.

R Soll is a Co-ordinating Editor for Cochrane Neonatal; however, he did not participate in the editorial process for this protocol.

M Bruschettini is a Senior Editor for Cochrane Neonatal; however, he did not participate in the editorial process for this protocol.

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Registration and protocol

Cochrane approved the proposal for this review in February 2024.

Data, code and other materials

As part of the published Cochrane Protocol, the following is made available for download for users of the Cochrane Library: search strategies.

Data sharing is not applicable to this article as it is a protocol, so no datasets were generated or analysed.

As part of the published Cochrane Review, the following will be made available for download for users of the Cochrane Library: full search strategies for each database; full citations of each unique report for all studies included, ongoing or waiting classification, or excluded at the full text screen, in the final review; study data, including study information, study arms, and study results or test data; consensus risk of bias assessments; and analysis data, including overall estimates and settings, subgroup estimates, and individual data rows. Appropriate permissions will be obtained for such use. Analyses and data management will be conducted within Cochrane's authoring tool, RevMan, using the inbuilt computation methods. Template data extraction forms from (51, Excel) will be available from the authors on reasonable request.

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