# The benefits and harms of whole genome sequencing newborn babies for rare diseases


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**Abstract**

**Background:** Rare genetic diseases are a group of disorders caused by alterations in a person's genes which can lead to various health problems. Of an estimated 7,000 rare disorders, about 80% are believed to have a genetic cause. Of an estimated 600 rare conditions that present before the 5th birthday, the UK is currently screening for nine with the newborn blood spot test. Whole genome sequencing (WGS) as a first-line screening test has emerged as a possible tool for the expansion of the newborn blood spot test.

Alongside many ethical considerations, WGS has several challenges which require thorough assessment: 1) Inferring presence of disease from genetic information requires a sound understanding of the link between genetics, gene products and disease. In rare diseases this link is often poorly understood;

2) Many genetic variations are unproblematic and even pathogenic variations do not result in symptomatic disease in all individuals (this is called incomplete penetrance). Understanding penetrance, i.e., how clinically defined disease correlates with underlying genetics is essential;

3) Individuals with symptoms can experience a range in severity. Therefore, careful selection of genetic variations to be included in a newborn screening programme is paramount to avoid overdiagnosis;

4) Defining disease by genetic markers rather than biochemical markers leads to a different spectrum of disease being identified and some disease may be missed;

5) Management pathways for pre-symptomatic disease may not be available and the benefits and harms of earlier treatment are unknown.

In this review we will address the question: What are the benefits and harms of whole genome sequencing newborn babies for rare monogenic diseases? The review is intended to help the UK National Screening Committee make a recommendation as to whether a newborn screening programme using WGS should be implemented.

**Approach:** A traditional review approach would consist of individual reviews for each possible condition with all their relevant gene variants to facilitate a decision around their inclusion or exclusion in a screening programme. This is not feasible due to the large number of conditions. However, there are currently no established methods to multi-indicator screening technologies, WGS being an extreme example as it can potentially target hundreds of conditions, each with an increasing number of gene variants believed to be pathogenic. The review will, therefore, approach the question in two parts.

1) We will undertake traditional evidence reviews of five conditions (selected to cover a range of scenarios that will impact the NHS) addressing six questions relating to penetrance, diagnostic accuracy, treatment effectiveness and harms. The five conditions are:
    - Pyridoxine-dependent epilepsy
    - Hereditary retinoblastoma
    - X-linked hypophosphataemic rickets
    - Familial hemophagocytic lymphohistiocytosis
    - Medium-chain acyl-CoA dehydrogenase deficiency (MCADD)

2) We will explore alternative approaches that may allow scaling up the review of WGS to hundreds of conditions by shortening the review process proportionate to the treatment requirements for the 5 conditions and by focusing on penetrance and actionability. This will include:
    - undertaking a review focusing on penetrance and actionability in newborn screening studies using WGS (to maximise benefits and minimise harms of screening by identifying subgroups of patients with most severe disease defined by gene variants with high penetrance and expressivity) not restricted to the 5 conditions but restricting to studies of WGS in newborn screening populations
    - building upon existing resources reporting actionability such as ClinGen - an open-access and centralised resource to define the clinical relevance and actionability of genomic variants.

The traditional reviews will be undertaken using a rapid evidence assessment approach producing an Evidence Summary, as described in the UK NSC guidance on evidence review process. We will compare conclusions from the traditional reviews with the findings from the alternative approaches to assess the feasibility of using an alternative approach to assess WGS for hundreds of conditions in the future.

Additionally, we will update an existing systematic review exploring the cost-effectiveness of WGS and WES (not limited to newborn screening use case) to explore two key methodological issues: 1) how the costs associated with WGS and WES have been estimated in existing economic analyses, and 2) what comparators have been included in existing cost-effectiveness analyses. This will provide a useful basis to think through an appropriate methodological approach to future modelling.

Finally, we will map approaches as to how WGS for newborn screening is being evaluated in other healthcare systems by reaching out to international committees and stakeholders.

Abbreviations

| | |
|---|---|
| C10 | Decanoylcarnitine |
| C8 | Octanoylcarnitine |
| CF | Cystic fibrosis |
| CHT | Congenital hypothyroidism |
| DBS | Dried Blood Spot |
| DNA | Deoxyribonucleic acid |
| EMS | Extended Mutation Screening |
| fHLH | Familial Haemophagocytic Lymphohistiocytosis |
| FP | False Positive |
| GA1 | Glutaric aciduria type 1 |
| HCU | Homocystinuria |
| HSCT | Haematopoietic Stem Cell Transplant |
| IVA | Isovaleric acidaemia |
| LRT | Lysine Reduction Therapy |
| MCADD | Medium-chain acyl coA dehydrogenase deficiency |
| MRM | Multiple Reaction Monitoring |
| MSUD | Maple syrup urine disease |
| NBS | Newborn Screening |
| NHS | National Health Service |
| NK | Natural Killer |
| P6C | piperideine-6-carboxylate |
| PDE | Pyridoxine-dependent epilepsy |
| PKU | Phenylketonuria |
| PLP | pyridoxal 5’-phosphate |
| PPIE | Patient and Public Involvement and Engagement |
| PPV | Positive Predictive Value |
| RB | Retinoblastoma |
| SCD | Sickle cell disease |
| UK | United Kingdom |
| (UK)NSC | (UK) National Screening Committee |
| WES | Whole Exome Sequencing |
| WGS | Whole Genome Sequencing |
| XLHR | X-linked Hypophosphataemic Rickets |
| α-AASA | Enzyme α-aminoadipic semialdehyde |

# 1 Background

## 1.1 Rare genetic diseases

Rare diseases are a group of disorders that are characterised by their relatively low prevalence in the population and are typically defined in the UK as affecting less than 1 in 2,000 individuals [1]. There are approximately 7,000 rare disorders, which combined affect around 6% of the population in the Western world [2]. About 80% of rare disorders are thought to have a genetic cause[2], i.e. are caused by alterations in a person's genes which can lead to various health problems. If a disorder is caused by an alteration (variation) in a single gene, this disorder is termed a monogenic disorder.

The impact of DNA variants is highly variable: variants can be benign or pathogenic or be of unknown significance. Resulting protein changes can have localised or systemic impact and can cause a spectrum of disorders with varied symptoms and complications, ranging from mild to severe or life-threatening. Disorders caused by genetic variants can present at any time, from birth (e.g., cystic fibrosis) to much later in life (e.g., Huntington's disease). It is estimated that there are about 600 childhood onset conditions for which there is a potential intervention [3].

Early detection of these conditions can be crucial. Early diagnosis enables surveillance and early intervention when available, which can significantly improve outcomes, and is particularly important in conditions with rapid progression and/or that cause irreversible damage. Due to their rarity, diagnosing and treating these conditions can be challenging.

We will consider the following five rare childhood onset genetic conditions in this review:

- Pyridoxine-dependent epilepsy
- Hereditary retinoblastoma
- X-linked hypophosphataemic rickets
- Familial hemophagocytic lymphohistiocytosis
- Medium-chain acyl-CoA dehydrogenase deficiency (MCADD)

The rationale for choice of these five conditions is detailed in the Methods.

### 1.1.1 Pyridoxine-dependent epilepsy

#### *1.1.1.1 Background*

Pyridoxine-dependent epilepsy (PDE or PDE-ALDH7A1, henceforth PDE, OMIM 266100) is a rare inherited form of epilepsy, mostly caused by variants in the ALDH7AI gene. Other forms not considered here include PDE-PNPO and PDE-PLPHP [4]. The ALDH7A1 gene is responsible for production of the enzyme alpha aminoadipic semialdehyde (α-AASA) dehydrogenase, which is involved in the breakdown of lysine in the brain. Deficiency of α-AASA dehydrogenase results in the accumulation of metabolites including piperideine-6-carboxylate (P6C), which in turn inactivates pyridoxal 5'-phosphate (PLP), the active form of pyridoxine. PLP depletion is thought to contribute to the epileptic features observed in PDE and treatment with large daily doses of pyridoxine (vitamin B6) leads to adequate seizure control for most patients. Neurotoxic metabolite accumulation associated with α-AASA dehydrogenase deficiency has also been purported to contribute to intellectual disability and developmental delay which occurs in as much as 75% of cases of PDE. In some individuals with PDE, however, the cause is unknown [5].

#### *1.1.1.2 Natural history*

Classic PDE usually presents during the neonatal period [6] with prolonged seizures that are difficult to control with anti-seizure medication; in 75% of cases seizures may occur within the first few hours of life. These seizures last several minutes and involve loss of consciousness, spasticity and convulsions. If untreated, periods of encephalopathy are common (irritability, crying, fluctuating tone, poor feeding). In some cases, affected individuals do not experience seizures until they are 1 to 3 years old (late-onset PDE). Intellectual disability and developmental delay are often present (around 75% of cases), especially in those with classic PDE [5,7,8]. Due to the symptoms of PDE being similar to other, more common neonatal disorders, the condition can be missed, and this has in some cases led to death [9].

#### *1.1.1.3 Genetics and epidemiology*

PDE is an autosomal recessive condition usually caused by a homozygous or compound heterozygous varianttion in the ALDH7A1 gene on chromosome 5q23 [10]. Over 165 pathogenic variants of ALDH7A1 have been identified and the vast majority are biallelic [7]. A recent review reported prevalences of PDE based on clinical diagnosis, to, as low as 1:396,000 in the Netherlands, and 1:783,000 in the United Kingdom [11]. However, these early studies only included patients who responded to a pyridoxine trial and are therefore likely to have under-estimated prevalence; more recent studies estimate the prevalence of PDE as 1:64,352 [11]. Children born to couples who are both carriers of the varianttion have a 25% risk of developing PDE[8,12].

#### *1.1.1.4 Screening and Diagnosis*

PDE should be considered when investigating intractable seizures in patients aged three and under. Historically, diagnosis was ascertained by a positive clinical response to pyridoxine treatment [13], however, techniques to diagnose PDE now include measurement of biomarkers in the urine, blood, or cerebral spinal fluid (i.e. $\Delta^1$-piperideine-6-carboxylate (or Δ1-P6C) α-AASA) [13] and genetic testing for pathogenic (or

likely pathogenic) variants in ALDH7A1 [7]. A 2020 consensus guideline recommended the use of α-AASA or Δ1-P6C as diagnostic biomarkers of PDE, either alone or in combination with "other biomarkers" but did not make specific recommendations about levels that should be considered diagnostic [7]. A 2007 series of 11 patients with definite, probable or possible PDE reported the control values used for both α-AASA and Δ1-P6C; α-AASA was above control values in 10 of 11 patients, while PA in plasma was elevated in all patients with elevated α-AASA levels [16,17] . Although MRI abnormalities have been reported [18], there are currently no imaging or electroencephalogram features that can confirm a diagnosis of PDE [16,19,20].

PDE is not currently screened for in newborn programmes in the UK.

#### *1.1.1.5 Treatment*

There is no cure for PDE. The mainstay of treatment has traditionally been daily, high doses of pyridoxine for seizure control. However, outcomes for patients are often still poor, even with early diagnosis. Adjunct lysine reduction therapies (LRT) (e.g., lysine restricted diet and arginine supplementation) aim to reduce the accumulation of metabolites thought to contribute to intellectual disability and developmental delay [21]. The combination of vitamin B6 and LRT is known as 'Triple Therapy' [22]. Small observational studies have suggested possible improvements in clinical outcomes from triple therapy however results are likely to be confounded by earlier age on initiation of treatment [23].

### 1.1.2 Hereditary retinoblastoma

#### 1.1.2.1 *Background*

Hereditary retinoblastoma (RB, OMIM 180200) is a rare embryonic malignant neoplasm of the eye. RB is caused by biallelic variants to the human retinoblastoma susceptibility (RB1) gene on chromosome 13q14 that codes for the RB protein [24,25]. Variants of the RB1 gene prevent production of functional protein leading to uncontrollable growth of cells in the retina, resulting in tumours [26]. It is estimated that 40 to 45% of all RBs are hereditary (resulting from a germline (transmitted) variant and a second variant occurring in retinal cell precursors); the remaining cases are sporadic (caused by two variants at the cellular level) [27]. Hereditary RB is usually bilateral (80% of cases), 5% are trilateral (including a pineal/midline neuroectodermal tumour) and 15% unilateral [26–28].

#### *1.1.2.2 Natural history*

Hereditary RB usually occurs at an average of 15 months of age [29] and may be picked up by targeted ocular screening before any symptoms develop if there is family history of the disease [30]. The most common first symptom of RB is leukocoria or visible whiteness of the pupil, which may be noticed in photographs taken using flash photography. Leukocoria was cited as primary reason for treatment referral in 62.8% of cases in a global cohort of 4351 patients, followed by strabismus (squint) in 10.2% and proptosis (protruding eye(s)) in 7.4% [31]. Other common symptoms include glaucoma and hypopyon (presence of pus), and if the tumour is large, the eye may become painful and inflamed [32]. High-risk features on presentation (e.g. optic nerve invasion) are more common with increasing age and are associated with poorer outcome [25]. If RB is left untreated, blindness can occur[33] and metastases will most likely develop[34]. In 5% of cases, heritable RB is associated with a midline brain tumour [35,36].

RB is considered to be largely curable, with 10-year survival rates from non-neoplastic causes no lower than the general population [37]. Survival rates have been shown to vary globally according to national income levels, however, with 3-year survival ranging from 99·5% (95% CI 98·8–100·0) for children from high income countries to 57·3% (52·1–63·0) for children from low-income countries [38]. Following curative treatment for hereditary RB, survivors have an increased risk of subsequent malignancy (standardised incidence ratio of 11.9, 95% CI 10.4, 13.5), with considerably higher risks for sarcoma of the bone or soft tissue [39].

#### *1.1.2.3 Genetics and epidemiology*

Hereditary RB is caused by a heterozygous germline variant on one allele and a second, somatic variant on the other allele of the RB1 gene on chromosome 13q14 [35]. Over 900 variants in RB1 have been reported [40]; research is ongoing to investigate whether the type of variant (e.g., nonsense, deletion, frameshift, or splicing variants) is associated with the clinical features of RB [38,41]. These variants are of very high penetrance and expressivity [25]. In 10-20% of cases, the variant is inherited from a parent who also has hereditary RB [40]. Children with one parent who has heritable RB have a 25% risk of developing RB (50% risk of inheritance and 90% penetrance) [42]. There are two known RB1 allele variants which show a parent-

of-origin effect; c.607+1G→T substitution and c.1981C→T (p.Arg661Trp) missense variant [43,44]. Otherwise, the variant in the affected child is new [42].

Around 44 cases of RB are diagnosed every year in the UK [24], and 40% of these are of the hereditary form [27]. There is a slightly higher incidence of bilateral RB amongst males [45–47]. In around 5% of people with RB, the part of chromosome 13q that contains the RB1 gene is missing. This rare form of RB is classed as hereditary and is known as chromosome 13q deletion [48].

#### *1.1.2.4 Screening and Diagnosis*

Many countries offer targeted ophthalmological screening for RB in children born into families where there is a history of RB [27]. Family members may have already undergone genetic counselling and testing, particularly where a germline RB1 variant was identified in the original proband, and genetic testing is likely to be offered to offspring of those identified as having a familial RB1 variant [24,27]. Subsequent ophthalmological screening of those identified as at risk of developing RB is usually based around red reflex testing [28], begins after birth and, may be repeated every few months until the child is 5 years old [24]. Children with dim or absent red reflex are referred to a specialist ophthalmology service for eye examination under general anaesthetic [49]. Unlike other cancers, RBs can be diagnosed by their appearance so a biopsy is usually not necessary. After RB is diagnosed, other tests are conducted to stage the tumour. These can include an ultrasound or MRI scans, a lumbar puncture, a bone marrow sample or a bone scan [50]. For those with bilateral or multifocal RB (hereditary), alterations in the RB1 gene can usually be detected in blood samples. For children with unilateral RB, genetic testing can clarify whether the disease is hereditary or somatic [51].

#### *1.1.2.5 Treatment*

Management of RB is complex and treatment regimens must be tailored dependent on the circumstances [52–54], including factors such as tumour stage, number of foci, localization and size of the tumour(s) [54]. Treatment options include enucleation, cryotherapy, laser treatment, chemotherapy or radiotherapy [51]. Small, localised tumours can be successfully treated with laser treatment or cryotherapy, however chemotherapy is often needed for more advanced cases or when RB is present in both eyes, as is often the case for the hereditary form of the disease. Chemotherapy has been shown to lead to tumour control and avoidance of enucleation (eye removal) or external beam radiotherapy in over 90% of patients with no evidence of seeding (tumour invasion) into the subretinal space or vitreous cavity prior to commencing treatment [55]. In a series of 869 eyes (540 patients) undergoing chemotherapy for RB, a total of 161 (19%) underwent enucleation at a mean of 15 months (range 1 to 191 months) [56].

Sometimes, enucleation must be performed [57,58]. According to the NHS, there is a high chance the child will lose some or all vision in the affected eye therefore successful treatment is highly dependent on identifying RB early. A UK retrospective case study of patients with bilateral retinoblastoma identified visual impairment in 38% (14/44) of children (i.e., Snellen acuity between 20/40 and 20/200 in the better eye) and legal blindness in 19% of children (vision of 20/200 or worse in the better eye) following chemotherapy [59].

### 1.1.3 Medium Chain Acyl-CoA Dehydrogenase Deficiency (MCADD)

#### *1.1.3.1 Background*

Medium chain Acyl-CoA Dehydrogenase Deficiency (MCADD, OMIM 201450) is an inherited metabolic disease in which medium-chain fatty acids cannot be oxidised. This leads to an accumulation of fatty acids in the body and to a disruption in energy production mechanisms, particularly that of ketone synthesis. People with MCADD thus cannot mobilise energy stores in periods of increased metabolic demand (i.e., fasting, intense exercise, illness, etc.) which sends them into a state of metabolic crisis.

A deficiency of MCAD can result in a build-up of acylcarnitines (esters that bind to fat molecules to transport them into mitochondria) in blood, which may be observed in patients with biochemical testing [60–62].

#### *1.1.3.2 Natural history*

MCADD typically presents in the first 2 years of life with hypoglycaemic episodes concurrent with illness or increased periods of fasting (i.e., with the reduction of night-time feeds). Severe hypoglycaemic episodes may lead to seizure, and metabolic decompensation characterised by vomiting, coma and even death. MCADD accounts for around 1% of Sudden Infant Death Syndrome / Sudden Unexpected Death in Infants [63–66], though the inclusion of MCADD in screening programmes has greatly reduced this [67]. Mortality following a metabolic crisis episode in undiagnosed people with MCADD is around 20% [68].

#### *1.1.3.3 Genetics and epidemiology*

MCADD is an autosomal recessive condition affecting the ACADM gene. In the majority of cases (>80%), the condition is caused by a homozygous 985A→G variant [69]. It is more prevalent in Caucasian populations and has a prevalence of 1 in 10,000 in the UK [69,70]. Other variants are more common in other ethnic groups (e.g., Japan [71]). In the UK, the prevalence of homozygous 985A→G carriers is estimated at 6.2 per 100,000 [69].

#### *1.1.3.4 Screening and Diagnosis*

In the UK, MCADD has been part of the standard blood spot screening battery since 2009 [adapted from 71]. The current testing and diagnostic pathways are presented in Figure 1. The current approach to screening for MCADD consists of measuring the concentration of acylcarnitines (primarily C8 and C10) in the blood. A raised level of these markers (C8 > 0.5 µmol/L and C8:C10 ≥ 1 [72]) is suggestive of an incomplete breakdown of medium-chain fatty acids due to MCADD. In the current screening and diagnosis pathway, positive metabolite findings trigger genetic testing looking for the common 985A→G variant in the first instance and followed by an extended variant screening for patients who do not have a homozygous 985A→G variant. The sensitivity of the screening programme for MCADD in England is estimated to be 94% [73].

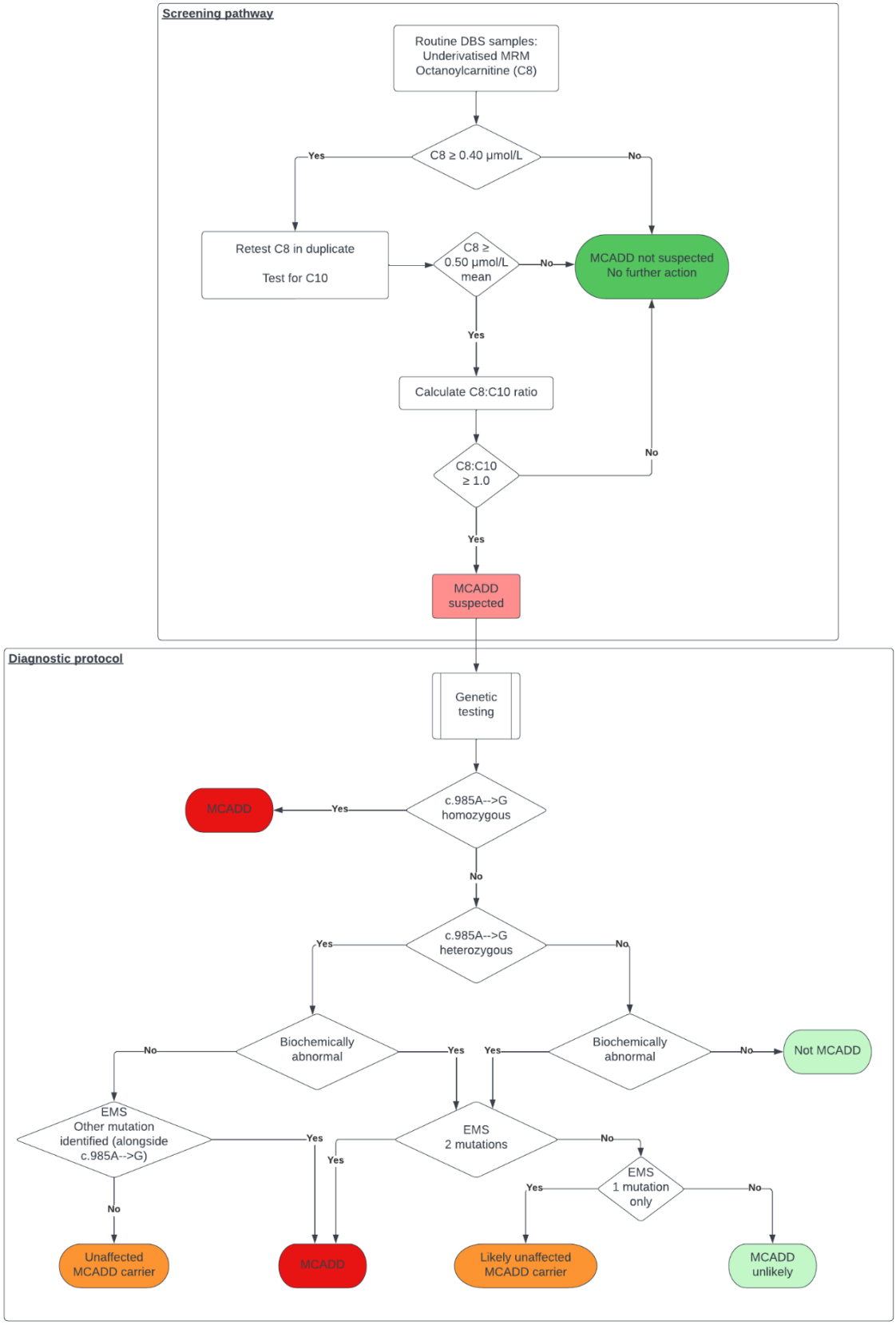


**Figure 1.1 Screening and Diagnostic pathways for MCADD** [74]

Key: C8: Octanoylcarnitine; C10: Decanoylcarnitine; DBS: Dried Blood Spot; EMS: Extended Mutation Screening; MCADD: Medium Chain Acyl-CoA Dehydrogenase Deficiency; MRM: Multiple Reaction Monitoring

#### *1.1.3.5 Treatment*

There is no cure for MCADD, but it can be effectively managed through diet. MCADD management typically consists of preventing hypoglycaemic episodes through limiting fasting periods [75,76]. Normal diet composition is normally acceptable, with the exception of coconut and coconut-derived products [77]. Diets should include sufficient complex carbohydrate intake, especially before fasting periods (i.e., night-time).

Acute illness increases risk of metabolic crisis. Emergency plans include prevention or treatment of hypoglycaemia through intake of fast carbohydrate by mouth or intravenous glucose infusion [78].

### 1.1.4 X-linked hypophosphataemic rickets

#### *1.1.4.1 Background*

X-linked hypophosphataemic rickets (XLHR, OMIM 307800), also known as X-linked hypophosphataemia, X-linked rickets, or vitamin-D resistant rickets, is a hereditary disorder of phosphate processing that causes a form of rickets. This is primarily characterized by osteomalacia (soft bones) and associated complications (bone deformity, bone and joint pain, dental problems). XLHR was first reported in 1957 and changes to the PHEX gene identified as a cause in 1995 [79,80].

The pathophysiology of XLHR is not fully understood but is known to primarily involve an increase in the FGF23 hormone. This triggers changes in both the kidneys and the parathyroid glands and ultimately results in increased renal phosphate wasting [81].

#### *1.1.4.2 Natural history*

Features of XLHR can be broadly divided into acute and chronic signs of hypophosphataemia. Signs of acute hypophosphataemia include muscle weakness, respiratory and cardiac insufficiency, neurological dysfunction, and blood disorder. Chronic signs include bone deformity, dental abscesses, stunted growth and bone and joint pain [78]. Clinical symptoms usually appear in the first two years of life, becoming more obvious with delayed walking or slowing down of growth/ exacerbation of leg bowing once toddlers become weight-bearing [82].

#### *1.1.4.3 Genetics and epidemiology*

XLHR is caused by a variant in the PHEX gene. Although most patients with XLHR have inherited a pathogenic variant from a parent, around 20% present with de novo variants, meaning that pathogenic changes to the gene have occurred spontaneously [83]. XLHR is the most common type of hereditary rickets [84,85], and penetrance is generally assumed to be 100% with no sex differences in penetrance [77]. Prevalence estimates range from 1.7:100,000 children to 4.8:100,000 children and adults [86].

#### *1.1.4.4 Screening and diagnosis*

X-linked hypophosphataemia is not currently screened for in newborn programmes in the UK. It is generally diagnosed in early childhood (usually before the 2nd birthday) through a combination of clinical features, biochemical characteristics, and radiological signs. Table 1.1 summarises some key features of X-linked hypophosphataemic rickets.

**Table 1.1. Key clinical, biochemical and radiological features of X-linked hypophosphataemic rickets**

| Clinical features | Biochemical characteristics | Radiological signs |
|---|---|---|
| - Short stature<br>- Leg bowing/knock-knees<br>- Delayed walking/abnormal gait | - Low serum phosphate*<br>- High urine phosphate | - Widening/cupping of metaphyses<br>- Rachitic rosary of ribs<br>- Sometimes “green stick” fractures |

| | | |
|---|---|---|
| - Dental abscesses | | |

*0-15 days: <5.6 mg/dL; 15-365 days: 4.8 mg/dL; 1-4 years: <4.3 mg/dL; 5-12 years: <4.1 mg/dL; 13-15 years: <3.2(female) / 3.5(male) mg/dL; 16-18 years: <2.9 mg/dL [87]

#### *1.1.4.5 Treatment*

There's no cure for X-linked hypophosphataemia, but it can be managed, with a goal of normalising serum phosphate concentration. This consists of oral supplementation of phosphorus (20 to 40 mg/kg/day) and calcitriol (active vitamin D, 20 to 30 ng/kg/day) multiple times a day [88]. This management approach is not always effective, but evidence suggests that earlier intervention is beneficial [77]. This strategy should include regular follow up to limit the risks of complications associated with treatment, which are commonplace. These include hypercalcaemia, hypercalciuria, kidney stones, nephrocalcinosis, impaired renal function and can lead to chronic kidney disease [89].

An alternative approach using burosumab injections was approved in the UK in 2018 [90]. Burosumab is an antibody against FGF23, which leads to an increase in renal phosphate reuptake/reduced wasting, increase in serum calcitriol, and increased gastrointestinal absorption of phosphate [91].

### 1.1.5 Familial haemophagocytic lymphohistiocytosis

#### *1.1.5.1 Background*

Familial haemophagocytic lymphohistiocytosis (fHLH) is an immunological disorder characterized by abnormal immune activation in which overactive macrophages target red blood cells. In terms of pathogenesis, fHLH involves a dysfunction of the cytotoxic perforin/granzyme pathway used by lymphocytes to target infected cells and downregulate the immune response as needed. The inability to neutralise overactive macrophages leads to an escalation of the immune response, including abnormal targeting of red blood cells and cytokine storms leading to organ damage.

#### *1.1.5.2 Natural history*

In fHLH, key downregulation mechanisms of the immune system are defective. Specifically, T-cells and Natural Killer (NK) cells have defective perforin/granzyme pathway (which is used by lymphocytes to trigger lysis of targeted cells, including overactive macrophages). This leads to a proliferation of lymphocytes and overactive macrophages which attack red blood cells causing anaemia. The cytokine storm associated with the unbridled immune response can lead to fatal multi-organ failure. fHLH usually manifests in infancy, with minor infections triggering an abnormal immune response. The prognosis for fHLH is poor but new treatments are promising [92].

#### *1.1.5.3 Genetics and epidemiology*

Different genes are associated with different types of fHLH [93]. fHLH is inherited in an autosomal recessive pattern [94]. Information pertaining to genes and pathophysiology of different types of fHLH are provided in Table 1.2. Here, the focus is on types of haemophagocytic lymphohistiocytosis that specifically involve the malfunction of the perforin/granzyme cytotoxic pathway.

**Table 1.2. Types of haemophagocytic lymphohistiocytosis affecting the perforin/granzyme pathway**

| HLH subtype (OMIM) | Gene involved | Pathophysiology |
|---|---|---|
| fHLH type 2 (OMIM 603553) | PRF1 | Affects perforin (a protein on the lytic granule that lets the granzymes into the target cells) |
| fHLH type 3 (OMIM 608898) | UNC13D | Affects munc13-4 (a protein that is involved in the fusion of the lytic granule and the target cell membrane) |
| fHLH type 4 (OMIM 603552) | STX11 | Affects syntaxin-11 (a protein involved in the docking of the lytic granule to the target cell) |
| fHLH type 5 (OMIM 613101) | STXBP2 | Affects munc18-2 (a protein involved in the fusion of the lytic granule and the cell membrane) |

There is little information about the prevalence of these disorders though they are rare [91]. The prevalence in Sweden is estimated to be 1.8:100k adapted from [95] and 1:100k in Texas [96].

### *1.1.5.4 Screening and diagnosis*

Familial HLH usually presents in infancy with symptoms including fever, enlarged spleen and liver, lymphadenopathy, and an array of neurological symptoms. Complications of fHLH can include anaemia, haemorrhage and secondary infection linked to decreased red blood cell, platelet, and neutrophil counts. These non-specific signs and symptoms associated with the rarity of fHLH can render diagnosis difficult. Classical fHLH laboratory findings can help with diagnosis, including high ferritin, abnormal cell counts, disturbed liver function markers [70]. Table 1.3 summarises the diagnosis criteria for fHLH.

**Table 1.3 Diagnostic criteria for familial haemophagocytic lymphohistiocytosis** [71,97]

| Diagnostic can be established if either A or B is fulfilled: | |
|---|---|
| A. Genetic variation consistent with fHLH | B. Any 5 of the following:<br>- Fever >38.5°C<br>- Splenomegaly<br>- Abnormal cell counts<br>  ○ Haemoglobin <9g/dL (<100g/dL for infants 4 weeks and under)<br>  ○ Platelets <100x$10^9$/L<br>  ○ Neutrophils <1.0x$10^9$/L<br>- High fasting triglycerides >3.0 mmol/L (>265mg/dL) and/or low fibrinogen (≤1.5g/L)<br>- Haemophagocytosis in bone marrow, spleen, liver, lymph nodes, or other tissue<br>- Decreased NK cell activity<br>- Ferritin ≥ 500µL<br>- High soluble IL-2 receptor ≥2,400U/mL |

### *1.1.5.5 Treatment*

Treatment of acute disease consists of supressing the immune response, through immunotherapy (i.e., corticosteroids) and chemotherapy but an important proportion of patients do not respond to these approaches. The only curative treatment currently available is allogeneic haematopoietic stem cell transplantation [98].

## 1.2 Current screening approach

In the UK, screening for rare diseases is conducted as part of the national newborn screening programme. Starting in the 1960s with screening for phenylketonuria, the programme has continued to grow since. In the early 2000s, the advent of tandem mass spectrometry marked a significant expansion as it allowed for the simultaneous testing of several metabolic disorders from a single dried blood spot [99]. Currently, the UK programme includes testing for 9 rare conditions (NHS website):

- Congenital hypothyroidism (CHT)
- Cystic fibrosis (CF)
- Sickle cell disease (SCD)
- 6 metabolic diseases:
    - Phenylketonuria (PKU)
    - Isovaleric acidaemia (IVA)
    - Glutaric aciduria type 1 (GA1)
    - Maple syrup urine disease (MSUD)
    - Medium-chain acyl coA dehydrogenase deficiency (MCADD)
    - Homocystinuria (HCU)

The newborn screening programme is very successful and has an uptake of more than 99%. This high level of participation is likely due to a combination of factors, including the recognition of the benefits of early detection of rare diseases among parents/caregivers, and the integration of the screening programme into routine newborn care.

Newborns who screen positive on the blood spot test undergo confirmatory testing. These include further biochemical tests only (e.g., PKU, MSUD) or could also involve genetic tests (e.g., CF, MCADD) [3,100].

## 1.3 Genetic testing

Genetic testing aims at identifying changes in chromosomes, genes or proteins to help confirm or rule out genetic disorders, or establish the likelihood of a person developing and passing on a genetic disorder in the future. Genes are segments of DNA that are transcribed into coding and non-coding RNA molecules. Coding RNA molecules are translated into proteins. Different forms – variants – of the same gene can exist, some of which can cause health problems – pathogenic variants. Genetic testing can target single variants or single genes either alone or as part of a gene panel, or can consist of Whole Exome Sequencing (WES, looking at all coding portions of the DNA) and Whole Genome Sequencing (WGS, looking at both coding and non-coding regions of the DNA).

### 1.3.1 Genetic testing in the UK newborn screening programme

Current approaches to screening for rare disorders have been successful in both their capacity for detection of infants with rare conditions, and in the wide uptake of the screening programme by the general public. However, the number of diseases currently screened for in the UK is limited and a number of factors must be taken into account for a new disease to be added to the screening panel (i.e., availability

and accuracy of test, pathways for testing, benefits of earlier diagnosis and treatment, cost-effectiveness, ethics, etc.).

In recent years, Whole Genome Sequencing (WGS) as a first-line screening test has emerged as a possible tool for an expansion of the newborn screening programme.

### 1.3.2 History of WGS

WGS is the process of determining the entire DNA sequence of an organism. Following pioneering work on DNA sequencing in the 1970s, the Human Genome Project (1990 - 2003) aimed at mapping the entire human genome. Since then, the development of Next Generation Sequencing technologies has dramatically increased the speed and decreased the cost of WGS, opening the door for its use in everyday clinical settings. Starting in 2013, Genomics England's 100,000 Genome Project focused on sequencing the DNA of patients with rare diseases and cancers and infections in the NHS with the view to improve diagnosis and treatment [101]. In 2021, Genomics England launched its generation study project aiming to use WGS to screen 100,000 newborns for rare diseases [102].

### 1.3.3 WGS for newborn screening

Expanding the newborn screening programme has been called for [103]. These calls included the benefits that WGS could potentially bring to newborn screening when used in tandem with the current bloodspot test. It has been argued that these benefits include: a large increase in the number of conditions identified (reducing diagnostic odysseys and facilitating earlier access to treatment and services), the possibility for increased efficiency of the programme (WGS would, in some instances, eliminate the need for repeat testing), and also through the notion of a lifelong genomic resource (a stored genome that could be 'dipped into' over the child's life course to guide diagnosis and treatment). Other suggested benefits have included reproductive benefit to parents and wider family, as well as the facilitation of registries and trials. Scaling up of the current blood spot test in its current form would by definition exclude the incorporation of disorders that do not have biomarkers detectable in blood and conditions for which biomarkers appear after the first few days of life, an issue potentially eliminated through WGS.

However, only 8 out of 10 rare diseases have a genetic cause identified [104] and not all genes associated with a specific genetic condition may be known. Moreover, many rare genetic conditions have wide spectrums of presentation and for some, it may not be possible to definitely distinguish between early and late onset forms of the condition by genotype alone (e.g. SMA, Pompe Disease) [105] with implications for treatment eligibility [106] and family wellbeing [107].

WGS could complement the newborn blood spot test because both tests could potentially detect different conditions.

#### *1.3.3.1 Challenges of WGS for newborn screening*

##### 1.3.3.1.1 Performance of genomic sequencing

WGS has been reported to have superior performance than WES and other DNA-based testing for diagnosing rare genetic diseases [108]. However, how WGS performs in newborn screening and how it

compares to current screening tests is largely unknown. A direct comparison of WES and tandem mass spectrometry showed that WES resulted in more false negatives and more false positives questioning its suitability as a first line test [109]. Decisions around the sensitivity-specificity balance skewing toward less false negative results will increase the rate of false positives leading to further testing, putting pressure on the referral pathways, increased use of healthcare services (Hayeems et al., 2017) and inflicting undue stress on families (Bendor-Samuel et al., 2023; Gurian et al., 2006).

#### 1.3.3.1.2 Ethical considerations

The use of WGS for newborn screening presents a number of ethical challenges. Firstly, since newborns cannot provide consent, NBS in the UK relies on obtaining informed consent from parent(s)/guardian(s). However, obtaining fully informed consent for WGS in the first week of a newborn's life could be challenging and tired parents may not feel that they can turn down screening [109,110]. As such, seeking consent for WGS, where large panels of conditions are screened for simultaneously, in the same manner may be inappropriate. Whether repeat consent should be sought periodically (i.e. if genetic data is stored for a long period of time, should consent be reiterated regularly?) as well as whether and when consent should transfer from the parent to the child must be considered [107]. There is also concern that WGS may have a negative impact on uptake rates for the NBS [109,110].

Secondly, a person's genome is a vast quantity of personal data. The duration of data retention, modalities for consent withdrawal and the process of data destruction need to be clarified. It has been suggested that genomic data can be retained, linked to health records, and re-evaluated throughout the person's life. Beyond the consent issues outlined above, this objective raises important questions about privacy and confidentiality, data ownership and secure data storage that need to be considered [111].

Thirdly, psychological implications may be associated with the decision to take part in or forgo screening [112], to anxiety around data security [113], through to communication of results [114], access to treatment and impact on the child and the whole family [115,116].

#### 1.3.3.1.3 Broadening the scope of newborn screening with WGS

Broadening the range of diseases screened has the potential for harms in the form of overmedicalisation and overtreatment, particularly for conditions in which penetrance is low (see below) and for conditions with a high number of variants of unknown significance. As noted by Horton and Lucassen (2022), most UK Biobank participants were found to have one or more rare non-synonymous variants when a panel to look at more than 500 disease genes was used [109,117,118]). These results, whilst initially alarming, reduce in their significance when viewed in light of the demographics of the population- primarily healthy adults over 45. As the authors note, had these results been produced in newborns, who had not yet had time to develop symptoms, the results would be more concerning, demonstrating the difficulties of an over reliance of genomic findings [119]. This difficulty of result interpretation, and the anxiety it can provoke are also not borne equally across social groups, and may be particularly exacerbated for people who are not of white European descent, who are underrepresented in reference data [120]. The reverse problem is also important; WGS may detect conditions for which no treatment is available. If more diseases are included in the newborn screening programme, adequately resourced referral pathways must be in place [121] which will present a challenge for resource allocation in health care systems. There is a resource trade-off

between early identification and intervention for less sick or asymptomatic individuals with a genetic diagnosis and resource-intensive diagnostic odysseys and later treatment when a rare disease was not screened for [122].

WGS for newborn screening will also raise questions around reporting of incidental findings (such as genetic predisposition to late-onset diseases, filiation information, etc) [123] and identification of carriers of pathogenic variants who are asymptomatic at the time of testing. This would lead to an increase in those living with chronic risk and uncertainty as 'patients-in-waiting' [124] or 'genetic nomads' [123].

#### 1.3.3.1.4 Genotype-phenotype relationship

Conditions are typically defined by signs and symptoms. Screening is undertaken in asymptomatic individuals to identify those with an increased risk of developing a condition. Gene variants and metabolite changes identified by screening, therefore, present risk factors for disease. Blood spot testing identifies a change in the amount or activity level of proteins including enzymes which may or may not lead to a person experiencing disease. Genetic sequencing identifies a change in DNA sequence which may or may not lead to a subsequent change in the amount or activity level of proteins (gene products). Inferring presence of disease from genetic or biochemical tests requires a sound understanding of the link between genetics, gene products and disease (severity). In other words, we want to be able to describe how a DNA variation, which alters the function or dosage of the resulting protein, links to a specific phenotype (i.e., the observable trait). However, in rare diseases this link is often poorly understood due to a lack of understanding of incomplete penetrance (see below) and variable expressivity (see below) of gene variants associated with a certain rare condition.

Penetrance is the proportion of individuals in a population that carry a specific gene variant as part of their genetic makeup (genotype) who go on to develop the corresponding phenotype (observable trait). Penetrance below 100% is called incomplete penetrance. This is very common due to a combination of genetic (e.g., disease resistant mechanisms, epigenetics and gene modifiers), environmental, and lifestyle factors [123]. The scope to investigate this for rare diseases is limited.

Expressivity is the degree to which a phenotype (observable trait) is expressed among individuals with the genetic variant resulting in a range in severity of symptoms. It is a qualitative measure and describes individual variability.

This means that screening to identify genetic variants that are known to be associated with a condition is not sufficient to predict the development/manifestation of disease. Healthy individuals have been shown to carry putatively pathogenic variants without ever developing clinical symptoms [125]. The understanding of the functional link between genotype and phenotype is essential for every rare genetic condition to inform the development of genomic screening programmes.

Investigation of the genotype-phenotype relationship, however, is complicated by the complexities of how monogenic disease is regulated and by incorrect variant association due to study design problems [126]. Ascertainment bias is particularly problematic because rare pathogenic variants have typically been studied 'in clinical cohorts carrying the disease often belonging to the same family, therefore displaying little variation. This has overestimated gene penetrance of many rare conditions by underestimating the

background variation in the gene in the population. Population based datasets of WGS information with associated clinical information on phenotypes are important for the investigation of penetrance and expressivity of rare disease variants [127–129]. Studies of such datasets have resulted in reclassification of previously reported pathogenic variants which were often based on single case studies. Furthermore, new variants are constantly identified adding to the flux in genetic variants linked to a specific condition.

#### 1.3.3.1.5 Selecting variants for reporting

Many genetic variants are unproblematic. The American College of Medical Genetics and Genomics (ACMG) produced standards and guidelines for the interpretation and classification of variants into five categories: "pathogenic," "likely pathogenic," "uncertain significance, "likely benign," and "benign" [130]. WGS for screening for rare diseases requires selecting pathogenic variants which should be reported to parents. This selection typically involves an assessment of 'actionability' of genes and gene variants [128]. It is a complex and time-consuming process which requires evaluation of the evidence on disease onset, severity of disease, likelihood of disease (penetrance), treatment effectiveness and burden of treatment for each gene variant-disease pair. Ongoing gene sequencing projects in newborns employ various approaches to assess actionability (Appendix 9.1). Berg et al. published an approach to identify clinically actionable incidental findings aiming for transparency and reproducibility [131]. They developed their approach in adults undergoing genome sequencing for other indications and adopted the approach to newborn screening [132]. Briefly, their approach is based on the principles of systematic reviews and consists of 3 stages [133]:

1) Applying rule out criteria to genes/conditions that do not meet a baseline threshold (5 questions in 3 pre-defined criteria on actionability, penetrance and association with a health condition)
2) Literature search with evidence synthesis into a short summary (5 criteria with several questions each, additionally considering significance/burden of disease, acceptability of intervention and risks)
3) A panel of experts reviews the short summaries for final selection.

The selected gene-condition pairs can then be scored using a semi-quantitative scoring method of 5 core characteristics on severity of disease, penetrance, efficacy of interventions, burden of intervention and knowledge base [134]. Each category has four levels with corresponding scores from 0-3. A score of ≥11 (the top quintile) was chosen as a threshold of actionability for reporting.

This approach was adapted by the Clinical Genome Resource (ClinGen) consortium [135–137]. ClinGen is a National Institutes of Health–funded consortium of researchers and clinicians who have built an open-access and centralised resource to define the clinical relevance and actionability of genomic variants [138]. They use a standardised protocol to produce summary reports and semi quantitative metric scores based on the approach by Berg et al. Each variant-condition pair is scored independently by multiple members and the scores are subsequently discussed using consensus for assigning a single actionability score. The summary reports provide ratings of the level of evidence that was available for each section of assessment. The database can be searched by gene name and provides a useful resource as it curates

evidence from several sources including published peer-reviewed literature, online resources such GeneReviews and databases including ClinVar and Decipher [99].

These published efforts illustrate that selecting gene variants for reporting in newborn screening using WGS requires a case-by-case assessment of each variant-condition pair. To evaluate newborn sequencing projects, the gene variant selection approach needs to be thoroughly understood.

#### *1.3.3.2 International initiatives for the use of genomics in newborn screening programme*

Screening for rare diseases has been part of standard newborn assessment batteries in many countries for the better part of the last 60 years. The number of diseases included in screening panels in different countries vary widely [139]. The increase in speed and decrease in cost of sequencing has raised the question of whether screening programmes could and should pivot towards genomic testing [88]. Consequently, many international pilots for the use of genomic testing in the context of newborn screening programmes are underway.

In 2022, the International Consortium on Newborn Sequencing was inaugurated with the aim of tracking progress of the research programmes that are being set up or are currently underway. Many of these studies are planning on using WGS or WES as a first line for their whole sample, while others will prioritise specified conditions using panel tests, and only use WGS/WES for confirmatory testing.

##### 1.3.3.2.1 US

Launched in the mid-2010s, the US-based BabySeq project (Massachussetts, US) led the way by evaluating the potential for genetic sequencing of newborn babies to diagnose, treat and monitor children with unanticipated monogenic disease risks. They followed up these children for 3-5 years to assess the impact of early access to genetic information and found that the detected genetic variants were sufficiently important to trigger action. In some cases, they explained existing health problems, in the rest, they informed about symptoms to watch out for. The implications were also significant for family members of the participants, some of whom were able to access surgeries to prevent cancer. BabySeq2 is now underway and aiming to recruit 1000 newborns from more varied ethnic backgrounds to determine their disease risks.

BeginNGS (California, US and Greece) is projecting to enrol 2,000 participants to screen for 500 disorders

Early Check (North Carolina, US) plans on enrolling 10,000 newborns to look at 200 childhood-onset conditions.

The GUARDIAN study (New York, US) aims to recruit 100,000 newborns over their 4-year project and are looking at around 250 conditions.

#### 1.3.3.2.2 Australia

BabyScreen+ (Australia) plans to recruit 1,000 babies for WGS to search for 500 treatable childhood onset conditions and builds on the back of the earlier project Baby Beyond Hearing which looked at genetic hearing loss.

Newborns in SA (Australia) aims to recruit 40,000 newborns into their WGS study.

#### 1.3.3.2.3 Europe

Screen4Care is large pan-European project launched in 2021 aims to enrol 18,000 newborns to look for actionable disorders and combine this with algorithm-based scanning of electronic health records to identify early symptoms of target conditions.

PERIGENOMED (France) plans to recruit 20,000 newborns for WGS.

In the UK, Genomics England is leading the newborn screening genomics initiative with the Generation Study launched in 2021 [140]. They are currently in the process of selecting conditions/genes for inclusion in the pilot programme and plan to start recruitment in late 2023. They aim to enrol 100,000 newborns into their study.

All these projects are largely in their development or early recruitment stages. Little information is currently available about methodologies used, particularly the approach of gene/condition selection. However, they appear to broadly stick to principles of actionability/treatability, and early childhood onset. More details about these studies where available are presented in appendix 11.1.

# 2 Research question and overall approach

## 2.1 Research question

The overall research question is:

**What are the benefits and harms of whole genome sequencing for the identification of rare monogenic diseases in newborn babies in the screening setting?**

## 2.2 Overall approach

A traditional review approach would consist of individual reviews of all proposed conditions with all possible causative gene variants (because WGS identifies gene variants rather than conditions) to allow a decision of inclusion or exclusion in a screening programme to be made for each variant. This traditional approach is not feasible due to the large number of conditions. However, there are currently no established methods to evaluate WGS for hundreds of conditions, each with a growing number of gene variants that are believed to be pathogenic. The review will, therefore, approach the question in two parts. A main review will consist of five traditional evidence reviews covering five conditions to establish the evidence base for a selection of conditions and to provide a reference case for comparison with alternative review approaches. We will then explore alternative approaches that may allow us to scale up the review by shortening the review process proportionate to the treatment requirements for the 5 conditions reviewed individual, and by focusing on penetrance and actionability. The latter will include:

Firstly, we will undertake a review of studies reporting penetrance in newborn WGS studies. The aim is to identify subgroups of patients likely to experience the most severe disease as defined by gene variants with high penetrance and expressivity to maximise benefits and minimise harms of screening. This will not be restricted to the five conditions.

Secondly, we will use existing resources such as ClinGen as a shortcut into the evidence on actionability for the genes associated with the five selected conditions to see if this can provide a similar answer to the main review approach (or reference case approach).

We will compare conclusions from the main review and the alternative approaches.

In addition, we will update an existing systematic review [141] exploring the cost-effectiveness of WGS and WES (not limited to newborn screening use case). We will focus the review on two key methodological issues: 1) how the costs associated with WGS and WES have been estimated in existing economic analyses, and 2) what comparators have been included in existing cost-effectiveness analyses. A cost-effectiveness analysis of WGS in newborn screening will be needed for a policy decision, and an overview of existing cost-effectiveness research and some clarity around these two methodological issues will provide a useful basis to think through an appropriate methodological approach to future modelling.

Finally, we will reach out to researchers and decision makers and other international committees to map approaches how WGS for newborn screening is evaluated in other healthcare systems and explore patient and public views on questions relating to evaluating and communicating WGS in newborns in addition to reviewing any public involvement in the research identified which we will be report using GRIPP2.

# 3 Evidence review of five conditions

The five evidence reviews will take the traditional systematic review approach. We will review the evidence by condition and consider all gene variants within the evidence base rather than undertaking reviews by pre-specified gene variants. This is because current evidence will not be consistent in variants reported and because of the anticipated lack of evidence for many variants particularly on gene penetrance. Furthermore, we expect current evidence to focus on conditions rather than subgroups of people with variants with greatest potential to benefit screening and intervention.

## Selection of five conditions for review

This review will focus on five monogenic conditions potentially suitable to be part of the WGS screening programme for newborns.

We have used a logical process to select conditions for the review that, whilst not fully representative of the many hundreds of conditions, does cover a range of scenarios that will impact the NSC's advice on whether WGS for screening newborns for rare conditions should be implemented. We developed a strategy for selection of monogenic conditions that maximises the chance of finding good evidence of benefit for screening. To this end we identified a shortlist of conditions which were considered by Genomics England to have met their four principles (GE score 1, judgement in July 2023) and for which the UKNSC had not previously reviewed and recommended against screening. To obtain a range of test and treatment consequences within the review we classified conditions on our shortlist according to whether the testing and treatment pathway is:

i. low cost to both NHS and patient/family
ii. centred around long-term surveillance with the associated anxiety and costs
iii. high and long-term cost to both patient/family and NHS
iv. short-term high costs to NHS but long-term lower costs to NHS and patients and where
v. existing screening and treatment pathways exist so impact of whole genome sequencing would be incremental.

We selected one condition at random which met our criteria from within each of the five categories stratified by disease area. This resulted in the following five conditions by category:

a) Pyridoxine dependent epilepsy: intervention is vitamin, disease area is neurology
b) Hereditary retinoblastoma: intervention is surveillance, disease area is cancer predisposition
c) X-linked hypophosphataemic rickets: intervention is monoclonal antibody burosumab (£2,992 per injection) injected once every 2 weeks for children 6 months to 17 years of age subcutaneously by a health care professional [142], disease area is endocrinology

d) Familial hemophagocytic lymphohistiocytosis: intervention is chemoimmunotherapy to treat active disease followed by allogeneic HSCT, the only curative therapy, disease area is immunology/haematology
e) MCADD (disease area is metabolic)

## 3.1 Decision questions

Decision questions for the traditional approach are shown in Table .

**Table 3.1. Key questions for the evidence summary, and relationship to UK NSC screening criteria**

| Key question* | NSC criterion |
|---|---|
| **Question 1.**<br>**What is the penetrance and expressivity of different gene variants associated with the conditions a), b), c), d) and e) in untreated infants/young people up to 18 years?** | 1.The condition should be an important health problem as judged by its frequency and/or severity. The epidemiology, incidence, prevalence and natural history of the condition should be understood, including development from latent to declared disease and/or there should be robust evidence about the association between the risk or disease marker and serious or treatable disease.<br><br>3.If the carriers of a mutation are identified as a result of screening, the natural history of people with this status should be understood, including the psychological implications. |
| **Question 2.**<br>**What proportion of infants/young people up to 18 years with**<br>**i. biochemical and**<br>**ii. biochemical and clinical**<br>**features* of conditions a), b), c), d) and e) carry the gene variants known for the conditions?** | 1.The condition should be an important health problem as judged by its frequency and/or severity. The epidemiology, incidence, prevalence and natural history of the condition should be understood, including development from latent to declared disease and/or there should be robust evidence about the association between the risk or disease marker and serious or treatable disease. |
| **Question 3.**<br>**What is the diagnostic accuracy (clinical validity) of gene sequencing for conditions a), b), c), d) and e)** | 4.There should be a simple, safe, precise and validated screening test.<br><br>8. If the test is for a particular mutation or set of genetic variants the method for their selection and the means through which these will be kept under review in the programme should be clearly set out. |

| | |
|---|---|
| **Question 4.**<br>**What is the evidence on early (following screen detection or sibling detection (cascade testing)) versus late (following clinical presentation) treatment?**<br><br>**If comparative data on early vs late treatment is unavailable, what is the treatment effectiveness in screen detected cases or following clinical presentation?** | 9. There should be an effective intervention for patients identified through screening, with evidence that intervention at a pre-symptomatic phase leads to better outcomes for the screened individual compared with usual care. Evidence relating to wider benefits of screening, for example those relating to family members, should be taken into account where available. However, where there is no prospect of benefit for the individual screened then the screening programme should not be further considered.<br><br>10. There should be agreed evidence-based policies covering which individuals should be offered interventions and the appropriate intervention to be offered. |
| **Question 5.**<br>**What is the effectiveness of WGS for newborn screening for conditions a), b), c), d) and e) to reduce disease-related morbidity and mortality (clinical utility) from best available evidence?** | 11. There should be evidence from high quality randomised controlled trials that the screening programme is effective in reducing mortality or morbidity. Where screening is aimed solely at providing information to allow the person being screened to make an "informed choice" (such as Down's syndrome or cystic fibrosis carrier screening), there must be evidence from high quality trials that the test accurately measures risk. The information that is provided about the test and its outcome must be of value and readily understood by the individual being screened. |
| **Question 6.**<br>**What are the harms of WGS for newborn screening for conditions a), b), c), d) and e) in terms of FPs, overdiagnosis, including variants of uncertain significance, ethics, anxiety, referral to surveillance pathway, missing management pathways, data storage, treating asymptomatic newborns and what are benefits beyond those from earlier treatment?** | 13. The benefit gained by individuals from the screening programme should outweigh any harms, for example from overdiagnosis, overtreatment, false positives, false reassurance, uncertain findings and complications. |

*Questions 1, 2 and 3 are related. Penetrance in this context is akin to the PPV in a test accuracy study. Question 2 is equivalent to half of a two-gate test accuracy study focusing only on cases and not controls. Test accuracy studies will additionally consider negatives (newborns without gene variants in prospective test accuracy studies and newborns without disease in two-gate test accuracy studies), so calculation of true negatives is possible which cannot be achieved by a combination of Questions 1 and 2.

## 3.2 Methods

The reviews will be undertaken using a rapid evidence assessment approach producing an Evidence Summary as described in the UK NSC guidance on evidence review process .

### 3.2.1 Identification and selection of studies

#### *3.2.1.1 Search strategy*

Systematic literature searches will be undertaken for each of the 5 conditions using terms for the conditions which will identify evidence for all review questions. The search strategies were developed in MEDLINE (Ovid) using terms relating to 1) Pyridoxine dependent epilepsy, 2) Retinoblastoma, 3) X-linked hypophosphataemic rickets, 4) Familial hemophagocytic lymphohistiocytosis and 5) MCADD.

The search will be adapted as appropriate for other bibliographic databases, these are likely to include; EMBASE (Ovid); Science Citation Index (Web of Science – Clarivate); The Cochrane Database of Systematic Reviews (Wiley), The Cochrane Central Register of Controlled Trials (Wiley). Examples of the search strategies that may be used in the major databases are provided in Appendix 11.2.

The search strategy will comprise the following elements:

1) Searching of electronic bibliographic databases,
2) Contacting experts in the field,
3) Scrutiny of references of included studies, relevant systematic reviews and genetics database (e.g. ClinGen) entries for each specific condition

For the review of cost-effectiveness studies, the searches used in an existing systematic review will be replicated (where possible) and run from July 2016 (the date up to which the original search was run) to identify new studies. Databases including EMBASE (Ovid), MEDLINE All (Ovid), Science Citation Index (Web of Science – Clarivate), EconLit, and the CEA Registry will be searched.

#### *3.2.1.2 Study eligibility criteria*

Studies that satisfy the following criteria listed in Table 3.1 will be included:

Table 3.1 Study eligibility criteria

| | |
|---|---|
| **Population** | Questions 1, 3, 5 and 6<br>Newborn babies without symptoms or known family history of the 5 conditions (newborn screening cohorts) or an approximation if evidence is limited (e.g. siblings without symptoms) (for question 6 approximations may be very wide as evidence is expected to be minimal)<br><br>Question 2<br>Newborns, children and young people up to 18 years (or an approximation) with clinical features or biochemical and clinical features*of the 5 conditions:<br>*Pyridoxine-dependent epilepsy*<br>Clinical: intractable seizures that respond to pyridoxine treatment,<br>Biochemical: multiple biomarkers (mainly $\alpha$-AASA, pipecolic acid) in the urine, blood, or cerebral spinal fluid<br>*Hereditary retinoblastoma*<br>Clinical: positive eye examination (indirect ophthalmoscopic examination with scleral indentation) usually following dim or absent red reflex testing<br>*X-linked hypophosphataemic rickets*<br>Clinical: bone deformity, dental abscesses, stunted growth and bone and joint pain<br>Biochemical: low serum phosphate, high urine phosphate<br>*Familial hemophagocytic lymphohistiocytosis*<br>Clinical: fever, enlarged spleen and liver, lymphadenopathy, an array of neurological symptoms<br>Biochemical: high ferritin, abnormal cell counts, disturbed liver function markers (see table 1.3)<br>*MCADD*:<br>Clinical: hypoglycaemic episodes characterised by seizure and metabolic decompensation (vomiting, coma, death)<br>Biochemical: C8 acylcarnitine levels ≥0.5µmol/L and C8:C10 ratio ≥1.0 or<br><br>Question 4<br>Early treatment: screen detected (any test) newborns or an approximation: 1) sibling detection, 2) other up to age 18 years who are positive for pathogenic variants (as defined by the studies) of the genes described for question 1.<br>Late treatment: Symptomatic detected (without screening) newborns, children and young people up to the age of 18 for the 5 conditions or an approximation (anything else available). |

| | |
|---|---|
| **Target condition** | All questions:<br><br>Pyridoxine dependent epilepsy,<br>Hereditary retinoblastoma,<br>X-linked hypophosphataemic rickets,<br>Familial hemophagocytic lymphohistiocytosis or<br>MCADD<br>defined by biochemical characteristics (or equivalent follow-up tests) or clinical symptoms as defined above |
| **Exposure / Intervention** | Questions 1, 2 and 3<br>Pathogenic variants (as defined by the studies) of the genes below identified by WGS using any technology, e.g. Sanger sequencing or Next Generation Sequencing (NGS) (step down; other genetic sequencing methods)<br>• ALDH7A1 (Pyridoxine dependent epilepsy),<br>• RB1 (Hereditary retinoblastoma),<br>• PHEX (X-linked hypophosphataemic rickets),<br>• PRF1, UNC13D, STX11, STXBP2 (Familial hemophagocytic lymphohistiocytosis) and<br>• ACADM (MCADD)<br><br>Question 4<br>Management following pre-symptomatic detection of the five conditions or symptomatic treatment if no evidence on pre-symptomatic treatment is available:<br>• Pyridoxine dependent epilepsy: Vitamin supplements of Pyridoxine<br>• Hereditary retinoblastoma: eye examinations, chemotherapy, laser treatment, cryotherapy, radiotherapy and surgery<br>• X-linked hypophosphataemic rickets: supplements of oral phosphate, active vitamin D, monoclonal antibody Burosumab<br>• Familial hemophagocytic lymphohistiocytosis: chemoimmunotherapy and allogeneic Hematopoietic stem-cell transplantation (HSCT)<br>• MCADD: dietary advice<br><br>Questions 5 and 6<br>Screening using WGS |
| **Comparator** | Questions 1, 2 and 3<br>No comparator<br><br>Question 4<br>Treatment following symptomatic detection or no comparator if no evidence on early versus late treatment is available<br><br>Question 5 and 6<br>No whole genome sequencing, current UK practice which is screening for 11 different rare diseases using tandem mass spectrometry after newborn blood spot screening, no comparator |

| | |
|---|---|
| **Outcomes** | Questions 1, 2 and 3<br>i) biochemical features associated with each condition, and<br>ii) biochemical and clinical features leading to a diagnosis of each of the 5 conditions as described above*<br>For Q3, each combination of biochemical and clinical features will be considered the 'reference standard' for calculation of diagnostic accuracy.<br><br>Question 4<br>Disease specific morbidity and mortality<br><br>Question 5<br>Any health-related health outcomes that can be measured across conditions (e.g. quality of live, mortality)<br><br>Question 6<br>Harms reported by studies including false positives, overdiagnosis, variants of uncertain significance, anxiety, data storage breaches, adverse events of early treatment, benefits beyond accuracy and patient outcomes (e.g. greater certainty (doctors and patients), reduced anxiety, fewer investigations, appropriate surveillance/management plan (therapeutic yield), earlier diagnosis, earlier treatment |
| **Study designs** | Question 1<br>Order of priority: Systematic reviews, observational studies of newborn screening population without treatment and follow up to disease, observational studies of screening population with treatment and follow up to disease with matched comparator, observational studies of screening population with treatment and follow up to disease and no comparator, observational studies of screening of which only gene variant positives are included, sibling studies, case series (i.e. more than one case or family)<br><br>Question 2<br>Order of priority: Systematic reviews, observational studies, case series<br><br>Question 3<br>As in question 1 plus case control studies<br><br>Question 4<br>Systematic reviews, randomised controlled trials, before and after studies and other cohort studies<br><br>Question 5 and 6<br>Systematic reviews, randomised controlled trials, non-randomised trials, before and after studies |
| **Language** | English language |

*This applies to conditions with biochemical features. Conditions, such as retinoblastoma, have no biochemical features and risk can therefore no be assessed with a biochemical test following a positive genetic test. At the time of screening and for some time afterwards these babies will have nothing other than the genotype. Q2 will be restricted to individuals with clinical features only.

Papers that fulfil the following criteria will be excluded:

Studies of people older than 18 years at diagnosis, studies of non-hereditary forms of the five conditions, qualitative studies, studies that provide insufficient information for assessment of methodological quality/risk of bias, studies reporting outcomes not listed in our inclusion criteria, studies where more than 10% of the sample do not meet our inclusion criteria and are not reported separately, articles not available in the English language, single case studies (studies of one case or one family; however, we will report the number of case studies per condition), letters, reviews, editorials, communications, conference abstracts, and other grey literature, publications that contain no numerical outcomes data.

### 3.2.2 Review strategy

Titles and abstracts of records identified by the searches will be screened by one reviewer. A second reviewer will independently assess a random 20% sample of the titles/abstracts. Disagreements will be resolved by consensus, or through discussion with a third reviewer. Full text articles will be assessed against the inclusion/exclusion criteria by one reviewer, with a random 20% sample assessed independently by a second reviewer. Disagreements will be resolved by consensus, or through discussion with a third reviewer. Records rejected at full text stage (including reasons for exclusion) will be documented.

### 3.2.3 Data extraction strategy

Data will be extracted by one reviewer, with a random 20% checked by a second reviewer. All data extraction will be entered into a piloted electronic data collection form. Any disagreements will be resolved by consensus or discussion with a third reviewer.

### 3.2.4 Assessment of study quality

The quality appraisal tool used for each study type is reported in Table 3.2 below. Quality appraisal will be conducted by one reviewer, with a random 20% checked by a second reviewer. For each question, quality appraisal will only be conducted for studies that employ the highest priority design available (see Table 3.1 for priority of study design by research question).

Table 3.2 Quality appraisal tools by study type

| Study type | Tool |
|---|---|

| | |
|---|---|
| Systematic reviews | Risk of Bias in Systematic Reviews (ROBIS) tool [142] |
| Cohort studies | Quality in Prognostic Studies (QUIPS) tool (question 1) [143]<br>Joanna Briggs Institute (JBI) Checklist for Cohort Studies (questions 2 and 4) [144]<br>Quality Assessment of Prognostic Studies (QUAPAS) (question 3) |
| Case-control studies | Joanna Briggs Institute Checklist for Case Control Studies [126]<br>Quality Assessment of Prognostic Studies (QUAPAS) (question 3) |
| Randomised trials | Cochrane Risk of Bias tool 2 (RoB-2) [145] |
| Non-randomised controlled trials | Risk Of Bias In Non-randomised Studies - of Interventions (ROBINS-I) [139] |

### 3.2.5 Methods for analysis/synthesis

We will employ an order of priority approach to analysis/synthesis. We will provide a narrative summary of studies that employ the highest priority design available only. For studies that employ lower priority designs we will provide details of the characteristics of the studies, including the study design, countries in the studies took place, sample sizes, and list the key outcomes in a table. The order of priority by study type is provided in Table 3.1.

**Question 1.** What is the penetrance and expressivity of different gene variants associated with the conditions a), b), c), d) and e) in untreated infants/young people up to 18 years?
For dichotomous outcomes, we will report the numerators, denominators, and proportion of participants with (1) any of the outcomes of interest, and (2) for each of the outcomes of interest. For continuous outcomes, we will report the mean and/or median. Data will be reported separately by condition and stratified by gene variant, different definition of disease for penetrance (biochemical or clinical), the country in which the study took place, and participant age, sex, and ethnicity.

**Question 2.** What proportion of infants/young people up to 18 years with
i. biochemical and
ii. biochemical and clinical
features (see * under table 1.3) of conditions a), b), c), d) and e) carry the gene variants known for the conditions?

We will report the numerators, denominators, and proportion of participants with any of the variants reported by the study. This will be reported by (1) any of the exposures of interest, and (2) for each of the exposures of interest. Data will be reported separately by condition and stratified by the country in which the study took place, and participant age, sex, and ethnicity.

**Question 3.** What is the diagnostic accuracy (clinical validity) of gene sequencing for conditions a), b), c), d) and e)
We will report true positive, false positives, true negatives and false negatives and calculate sensitivity and specificity as well as predictive values separately for the five condition and stratified by gene variant and different reference standard (biochemical or clinical features).

**Question 4.** What is the evidence on early (following screen detection or sibling detection (cascade testing)) versus late (following clinical presentation) treatment? If unavailable, what is the treatment effectiveness?
For dichotomous outcomes, we will report the numerators, denominators, and proportion of participants with (1) any of the outcomes of interest, and (2) for each of the outcomes of interest. For continuous outcomes, we will report the mean and/or median at the beginning and end of the study and change scores. Data will be reported separately for the conditions and stratified by timing of detection (pre-symptomatic versus symptomatic), and method of detection (screen detection, detection through sibling (cascade) testing, incidental detection, symptomatic presentation).

**Question 5.** What is the effectiveness of WGS for newborn screening for conditions a), b), c), d) and e) to reduce disease-related morbidity and mortality (clinical utility) from best available evidence?
For dichotomous outcomes, we will report the numerators, denominators, and proportion of participants with (1) any of the outcomes of interest, and (2) for each of the outcomes of interest. For continuous outcomes, we will report the mean and/or median at the beginning and end of the study and change scores. Data will be reported separately for the conditions and stratified by intervention/comparator pairs, e.g. genetic screening versus usual care, genetic screening versus phenotypic screening.

**Question 6.** What are the harms of WGS for newborn screening for conditions a), b), c), d) and e) in terms of FPs, overdiagnosis, including variants of uncertain significance, ethics, anxiety, referral to surveillance pathway, missing management pathways, data storage, treating asymptomatic newborns and what are benefits beyond those from earlier treatment?
For dichotomous outcomes, we will report the numerators, denominators, and proportion of participants with (1) any of the outcomes of interest, and (2) for each of the outcomes of interest. For continuous outcomes, we will report the mean and/or median at the beginning and end of the study and change scores. Data will be reported separately for the conditions and stratified by intervention/comparator pairs, e.g., genetic screening versus usual care, genetic screening versus phenotypic screening.

# 4 Methodological questions

A full review of 200+ individual conditions to evaluate WGS for newborn screening is not feasible. This review will explore ways to possibly scale a review of a few conditions to many conditions to enable the evaluation of WGS. (The traditional review will be the main review). We will explore shortening the review process proportionate to the treatment required for the five conditions reviewed in the main review. This

means exploring whether less evidence may be required for conditions with treatments that have low cost to patients and the NHS and more evidence for conditions with high-cost treatments.

## 4.1 Focusing the review on penetrance and/ or actionability

### 4.1.1 Approach and rationale

We will explore the feasibility of focusing the review on benefits of screening by targeting subgroups of patients with most severe disease as defined by gene variants with high penetrance and expressivity. Penetrance may be expressed as an aspect of actionability (the combined level of evidence regarding the pathogenicity and penetrance of a variant, the efficacy, burden, and availability of interventions, and the severity of potential disease [139]. Screening for conditions with more severe predicted disease will maximise benefit and decrease harms from screening. This could potentially be used to support a policy decision to implement WGS screening at a high threshold (low sensitivity), collect evidence and later decide whether the threshold can be lowered.

### 4.1.2 Research question

What is the penetrance or actionability of gene variants of rare genetic child-onset diseases identified in newborn screening populations using WGS?

### 4.1.3 Methods

#### *4.1.3.1 Identification and selection of studies*

Systematic literature searches will be undertaken combining terms relating to WGS and WES and newborn screening. If no eligible studies are identified, we will identify studies which represent a step down from our ideal study type and include broader terms of genetic testing to consider studies using panel testing and single gene testing. The search strategy will be fully developed in MEDLINE (Ovid) and adapted for other bibliographic databases.

#### *4.1.3.2 Eligibility criteria*

Studies that satisfy the following criteria listed in Table 4.1 will be included:

Table 4.1 Study eligibility criteria

This approach relies on the availability of information on penetrance / expressivity and generalisability of findings to the screening context. We will therefore only include studies of newborn screening populations that report as a minimum the penetrance (or an approximation) of gene variants linked to rare genetic diseases with childhood onset.

| | |
|---|---|
| **Population** | Newborn babies without symptoms or known family history of rare genetic diseases |
| **Target condition** | Any rare genetic condition with childhood onset of symptoms |

| | |
|---|---|
| **Exposure / Intervention** | Screening using WGS or WES<br>Step down: genetic testing using panel tests or single gene testing in newborns without symptoms or known family history |
| **Comparator** | Order of priority (comparator is only needed for approximation of penetrance using allele frequency):<br>1. No comparator necessary<br>2. Randomisation WGS vs standard care<br>3. Temporaneous cohort of matched / random newborns without genetic screening<br>4. General population of newborns without genetic screening<br>5. General population of healthy adults |
| **Outcomes** | For comparator 1: follow up without treatment to clinical features present, step down to follow-up biochemical tests indicating presence of disease processes<br><br>For comparator 2 to 5: Allele frequency in screened and comparator to approximate penetrance |
| **Study designs** | Order of priority:<br>Observational study of WGS of asymptomatic newborns without reporting to parents (no treatment) and follow-up to clinical features (or reporting results but no treatment until symptomatic, or reporting results and then later stopping treatment to determine whether necessary).<br>Observational study of WGS with follow-up testing (e.g. biochemical test)<br>Observational study of WGS follow-up to symptom onset despite treatment<br>Randomised trial of WGS (with results reported and treatment) vs standard care to determine allele frequency and approximate gene penetrance<br>Comparative studies using comparators detailed above to determine allele frequency and approximate gene penetrance |
| **Language** | English language |

Papers that fulfil the following criteria will be excluded:

Studies of populations other than newborns, studies on populations at risk or with symptoms, studies where WGS is second tier test, qualitative studies, studies only reporting variant frequency without an estimation of penetrance, studies that provide insufficient information for assessment of methodological quality/risk of bias, studies reporting outcomes not listed in our inclusion criteria, studies where more than 10% of the sample do not meet our inclusion criteria and are not reported separately, articles not available in the English language, single case studies (however, we will report the number of case studies per condition), letters, reviews, editorials, communications, conference abstracts, and other grey literature, publications that contain no numerical outcomes data.

#### *4.1.3.3 Assessment of study quality*

Study quality will be assessed using the quality appraisal tools listed in Table 3.2.

#### *4.1.3.4 Methods for analysis/synthesis*

We will report the numerators, denominators, and proportion of participants with the outcomes present (=penetrance) for each condition separately (as reported in the included studies), for all conditions combined per study and for potential categories (e.g., subgroups similar to Genomic England's categories with scores 1 to 3). If feasible we will determine the variant threshold (i.e., number and type of variants) that corresponds with the most severe phenotype for any conditions included in this review question.

We will narratively compare the results on penetrance with the results from question 1 from the main review for the 5 conditions and conclusions from this approach with the conclusion from the main review considering all five questions.

We will compare the results on penetrance with the actionability score on ClinGen for the conditions included in this review question and estimate the combined penetrance for those conditions that scored 10 or 11 out of 11 in actionability on ClinGen. We will report combined penetrance for any groups of conditions predefined by study authors in advance of data collection.

## 4.2 Using the ClinGen actionability score as evidence

The policy question around WGS for newborn screening will involve a decision on each variant proposed to be included in the new newborn screening programme. This is similar to the decision of actionability of gene variants in the ClinGen resource. ClinGen's expert review of the clinical relevance of genes and variants used standardised methodology [139] and provides relevant information for this review. We will explore using this resource for whether it can contribute to evidence considered by the UKNSC.

### 4.2.1 Methods

We will search the database for each of the genes included in the main review and identify reports from the paediatric actionability working group. For each of the 5 conditions in the main review we will compare the evidence identified by our review and referenced by ClinGen. We will assess the rating of the evidence reported in ClinGen and compare our conclusions to the actionability score for each variant. We will explore whether changing the threshold for actionability is feasible and useful for the NSC context.

## 4.3 Methods on costing WGS and comparators in cost-effectiveness studies

A cost-effectiveness analysis of WGS in newborn screening will be needed for a policy decision. A cost-effectiveness analysis of WGS of potentially hundreds of conditions will require different approaches to cost-effectiveness analyses of screening programmes for single conditions. One challenge is to attach

costs that need to apply to many different conditions with different patient pathways. A second challenge is the uncertainty around the complex and rarely linear diagnostic pathways for rare diseases with unspecific symptoms that need to be considered in the comparator of a cost-effectiveness analysis. We will produce an overview of existing cost-effectiveness research around WGS and WES considering two methodological issues to provide a useful basis to think through an appropriate methodological approach to future modelling. We will update an existing systematic review [146] exploring the cost-effectiveness of WGS and WES (not limited to newborn screening use case). We will focus the review on two key methodological issues: 1) how the costs associated with WGS and WES have been estimated in existing economic analyses, and 2) what comparators have been included in existing cost-effectiveness analyses.

### 4.3.1 Methods

Studies that satisfy the following criteria listed in Table 3.2 will be included in the cost-effectiveness review:

| | |
|---|---|
| Population | Studies in Human Healthcare |
| Intervention | Whole-genome and whole-exome sequencing (any platform) |
| Comparator | Any comparator |
| Outcomes | Costs |
| Study Design / publication type | Health technology assessments, systematic reviews, meta-analyses, economic evaluations, evidence-based guidelines, conference abstracts, clinical trials |

The eligibility criteria replicate those used in the Schwarze et al. review [147–151], except that we will not be including studies which only look at clinically relevant outcomes, or outcome studies, as we are interested in the costing methodology applied. Eight of the studies included in the Schwarze et al. [148] review were outcome studies and therefore will not be included in this review.

Given the focus of the cost-effectiveness review is the methodology used, rather than the risk of bias to the results, we will not appraise the quality studies included in that review.

For the studies meeting the eligibility criteria in the cost-effectiveness review, we provide a tabular overview of each study (in PICO form), the type of economic analysis conducted (e.g., budget impact, cost-effectiveness) and a brief description of the methodology adopted.

We will then focus on two key methodological questions:

1) How the costs associated with WGS and WES have been estimated
2) What comparators have been included in each study

For each of these questions, we will extract data and present it in a tabular form alongside a narrative summary.

# 5 Patient and Public Involvement

We will involve a group of approximately 8 people in the review who will either be adults with lived experience of rare genetic conditions, parents of children with rare genetic conditions, parents (with children five or under) or prospective parents/young people who would like to have children in the future or patient advocates working in the area of rare genetic conditions. The aim is to recruit people with different types of experience with genetic conditions (e.g., experience of early fatal conditions, sensory disability, mobility impairment, late onset) rather than by diagnoses, to have broad representation of views and experiences. The group will explore broad questions around the harms and benefits of genomic newborn screening bringing in their broader experiences of genetic testing rather than focusing on individual conditions. These will include:

- What are the key harms and benefits of WGS NBS that should be considered by policymakers and how should we detect/measure them?
- How should variants of uncertain significance be approached?
- What should be the minimum evidence on WGS available for decision makers (including their views on the adequacy of the evidence base, evidence limitations and gaps)?
- Should we have different standards of evidence for rare conditions compared to other, more common, conditions?
- How should we deal with conditions where penetrance is known from family studies but not from newborn screening populations?
- How do we explain complex genetic results to parents (e.g., penetrance and expressivity)?

The overarching aims of the Patient and Public Involvement and Engagement (PPIE) in this review are 1) to build an understanding of the broader societal views, perspectives and experiences of members of the rare disease community around the key challenges and opportunities of WGS NBS, 2) to broadly explore views towards, and understandings of, screening programmes and the role of the UK NSC, 3) to discuss methodological challenges identified during the review process, 4) to note views on limitations in the evidence base, and 5) to contribute meaningfully to the development of the protocol for the main review and health economic analysis for the NSC in a few years' time.

We will organise 5 2h-virtual meetings to discuss the questions posed which will be independent of the review stage. Meetings will be deliberative. We will present the knowledge and evidence base for the relevant question on the day to inform the group and create the deliberative knowledge space required for discussion, drawing on relevant evidence. Participants may also be given preparatory information/resources outside of meetings. We will seek consent to record all meetings, to collate PPIE views on WGS in newborn screening and build on the evidence base in PPIE involvement in WGS by understanding the wider contribution a PPIE group can make to the debate. These discussions will also help us understand how WGS and decisions around a new newborn screening programme need to be communicated to the wider population.

We will consider the ACTIVE framework to describe the PPI involvement where applicable [152].

# 6 Timescale & notes

**Table 6.1 Project timeline**

| | June 23 | July | Aug | Sept | Oct | Nov | Dec | Jan 24 | Feb | Mar | Apr | May | June | July | Aug |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Meetings with NSC June to August 23 to discuss research questions and review approach | | | | | | | | | | | | | | | |
| Protocol development | x | x | x | X final mid-sept | | | | | | | | | | | |
| Search strategy development | | | x | | | | | | | | | | | | |
| Finalise PICOS | | | x | | | | | | | | | | | | |
| Data extraction sheet development | | | x | x | | | | | | | | | | | |
| Tailoring of quality appraisal tools | | | | x | | | | | | | | | | | |
| Final protocol sign off by NSC and ESP | | | | x | | | | | | | | | | | |
| Prospero registration of protocol | | | | | x | | | | | | | | | | |
| Run searches for main review | | | | x | | | | | | | | | | | |
| Sifting T/A | | | | x | x | | | | | | | | | | |
| Retrieval of full texts | | | | | x | x | | | | | | | | | |
| Sifting FT, reference lists, related searches | | | | | | x | | | | | | | | | |
| Final selection of included studies in main review | | | | | | | x | | | | | | | | |
| Meeting with NSC in Dec to update on included studies | | | | | | | | | | | | | | | |
| Run searches for 2 methodological questions | | | | | | | | x | | | | | | | |
| Sifting T/A | | | | | | | | | x | | | | | | |
| Sifting FT | | | | | | | | | | x | | | | | |
| Data extraction | | | | | | | | x | x | x | x | | | | |

| | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Quality appraisal | | | | | | | | x | x | x | x | | | | |
| Synthesis | | | | | | | | | x | x | x | | | | |
| Search ClinGen | | | | | | | | | | x | | | | | |
| ClinGen results synthesis and comparison to main review | | | | | | | | | | | x | | | | |
| Meeting with NSC in April to update on study synthesis/findings | | | | | | | | | | | | | | | |
| Writing of introduction | | | | | x | x | x | x | x | | | | | | |
| Writing of report | | | | | | | | | | x | x | x | | | |
| PPI recruitment | | | | x | | | | | | | | | | | |
| PPI meetings | | | | | x | x | | x | | x | x | | | | |
| PPI write up | | | | | | | | | | | x | x | | | |
| Draft report to NSC | | | | | | | | | | | | Mid May | | | |
| Meeting with NSC in May to discuss draft report | | | | | | | | | | | | | | | |
| QA | | | | | | | | | | | | x | x | | |
| Final report to NSC / ESP | | | | | | | | | | | | | x | | |
| Prepare manuscript(s) for publication | | | | | | | | | | | | x | x | | |
| Adjust report with FMCH comments for consultation | | | | | | | | | | | | | x | x | |
| Responses to consultation comments (if required) | | | | | | | | | | | | | | | x |
| Responses to UK NSC feedback | | | | | | | | | | | | | | | ongoing |

## 7 Research team's contributions


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## 8 Competing interest of authors and advisors

Sian Taylor-Phillips (role on review: overall oversight of the review) is chair of the UK NSC Research and Methodology Group who have submitted the topic to the NIHR ESP for topic selection.

Jim Bonham is clinical advisor to Genomics England.

David Elliman is clinical advisor to the UK NSC and clinical lead to the NHS Newborn blood spot screening programme.

Graham Shortland is a member of the Ethics Task Group (part of Fetal Maternal Child Health expert group)

## 9 Acknowledgments

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# 11 Appendices

## 11.1 International WGS/WES newborn screening studies

| Project | Country | Key persons | Date | Number of babies, design | Sequencing approach, number of genes (variant list) | Notes |
|---|---|---|---|---|---|---|
| BABYSEQ™ | US | Robert C. Green<br>Ingrid Holm | 2015-2020 (BabySeq1)<br>2022-2025 (BabySeq2) | Randomised | WES<br>954 (no) | Clinicaltrials.gov ID: NCT02422511<br><br>**Key papers:** [152,153]<br><br>**gene selection approach** [127,130,131,154]<br>**4 attributes evaluated for inclusion of gene**:<br>- Validity of gene-disease association<br>- Age of onset<br>- Penetrance<br>- Inheritance<br>**Return criteria**<br>- Childhood onset<br>- At least moderate evidence and/or moderate penetrance<br>- Strong pharmacogenetic association<br>- Carrier status for any of the genes meeting the criteria<br>Based on results, **genes classified into 3 categories**:<br>- Cat A: genes included in the newborn genomic sequencing report with definitive strong evidence that it causes a highly penetrant childhood-onset disorder<br>- Cat B: Genes included in the report based on actionability during childhood |

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| | | | | | | Cat C: Genes that did not meet criteria (and thus not returned in the report) |
| BeginNGS™ | US / Greece | Stephen Kingsmore | ongoing | >2,000 Observational | WGS ~460 (yes) | **Key papers:** [130]<br><br>**Gene selection approach**, 6 phases [152,153]:<br>1. **Genome to treatment disorders:** genetic diseases pre-selected are<br>• Sufficiently severe to lead to ICU admission<br>• Can be Dxed by WGS<br>• Have effective treatments<br>2. **Newborn screening- rapid WGS list development:**<br>• Is natural Hx well understood?<br>• Significant risk for morbidity and mortality in young children?<br>• Effective and accepted intervention available?<br>• Does early Tx improve outcome?<br>• Benefits of intervention clearly outweigh risks?<br>• For genes with >1 associated disorders, do Tx differ? Can they be distinguished by WGS/another test?<br>3. **Development of structured curation system**<br>4. **Expert review**<br>5. **Roundtable discussion**<br>**Final inclusion/exclusion decision** |
| Early Check | US | Don Bailey<br>Holly L Peay | | 10,000 Observational | WGS ~200 (no) | **Condition selection approach**, from Early check website (*no longer accessible since study stopped recruiting in at some point in July 2023)<br>• Childhood onset<br>• Early diagnosis is difficult |

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| | | | | | | • Cause serious sickness, death, or has big impact on families<br>• Has low-cost proven lab test that can be performed on blood taken from a heel finick<br>• Has follow-up service available for affected children<br>• Has new treatments being developed that may be more effective when used early in life<br>Screening would lead to an overall benefit for babies and families |
| GUARDIAN STUDY | US | Wendy Chung | 2021-present | 100,000<br>Observational | WGS<br>160 (no) | ------ |
| SCREEN 4CARE | EU | Alessandra Ferlini | | 18,000<br>Observational | Panel (+ WGS for newborn testing negative but Sx in the first few months of life)<br>TBC (no) | Two sets of rare diseases, either<br>1. Treatable<br>2. Actionable |
| PERIGENOMED | France | | | 20,000<br>Observational | WGS<br>~150 | ------ |
| murdoch children's research institute Baby Screen+ | Australia | Sebastian Lunke<br>Zornitza Stark<br>Et al. | | 1,000<br>Observational | WGS<br>~500 (no) | ------ |
| NewbornsInSA | Australia | Karin Kassahn | 2023- | 40,000 | WGS | ------ |

| | | | | | | |
|---|---|---|---|---|---|---|
| Genomics england<br>Generation Study / Newborn Genomes Programme | UK | Richard Scott | 2023- | 100,000 Observational | WGS ~250 | **Gene selection approach**:<br>- Working groups<br>- Based on 4 principles for inclusion<br>  ○ Strong evidence for gene-disease link and reliably detectable<br>  ○ High penetrance/expressivity and debilitating phenotype<br>  ○ Early/pre-symptomatic intervention substantially beneficial compared to intervention after onset on symptoms<br>  ○ Intervention equitably accessible<br>- Literature review for each of the potential conditions<br>- Each gene categorized as either<br>  ○ Cat 1: gene/condition satisfies 4 principles outlined above<br>  ○ Cat 2: Unclear whether gene/condition satisfies the 4 principles --> expert input required<br>  ○ Cat 3: Gene/condition does not satisfy 4 principles and is childhood onset<br>  ○ Cat 4: Gene/condition does not satisfy 4 principles and is adult onset<br>- Programme focused on variants. If there is at least one variant in a gene which meets the four principles, the gene is taken forward.<br>- List of potential genes is sent to each chair of the relevant clinical reference group (CRGs) for views and consensus<br>- NHS Clinical Assurance Group provides assurance to the Programme and NHS on the selection of the right conditions and the |

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| | | | | | | availability and capacity of the downstream clinical pathways.<br>- Variants are drawn from external databases such ClinVar, publications, external variant curation, interpretation providers through competitive tendering.<br>- Variants linked to the included conditions that are pathogenic or likely pathogenic using ACMG and ACGS criteria are taken forward to the inclusion list. |
| | | | | | | |
| First 1,000 Days of Life Study | US | John E. Niederhuber<br>Joe Vockley<br>Kathi Huddleston | | 1,349 | WGS<br>329 | |
| NC NEXUS | US | Jonathan S. Berg | 2016-2019 | ~400 | WES<br>466 | **Key papers:** [127,130,131,154]<br><br>**Gene selection approach** [130]<br><br>1. Starting with gene list including conditions related to NBS as well as other genes previously curated by the group<br>2. Primary literature and online genetic resources used: GeneReviews, ClinVar, OMIM looking at<br>- Natural Hx<br>- Tx<br>- Interventions<br>- Evidence for gene-disease link |

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| | | | | | | 3. One reviewer scores each gene-condition pair<br>4. Committee discussion for consensus<br><br>Use a specifically designed measure: ASQM (Age-based Semi Quantitative Metric). Includes the following *<br>- Severity<br>- Likelihood<br>- Efficacy<br>- Acceptability<br>- Knowledge<br><br>All score on 0 to 3 scale, and adjusted for age criteria, categorized into *<br>1. NGS-NBS panel<br>2. Parental decision (peds onset of Sx but lower actionability)<br>3. Parental decision (adult onset Sx but high actionability<br>4. Not returned (adult onset, lower actionability) |

## 11.2 Proposed search strategies

### 11.2.1 Pyridoxine dependent epilepsy

**Ovid MEDLINE(R) ALL <1946 to September 18, 2023>**

| Concepts | # | Search terms | Hits |
|---|---|---|---|
| pyridoxamine dependent epilepsy | 1 | (exp vitamin b 6/ or exp pyridoxal/ or exp pyridoxamine/ or exp pyridoxine/) and (Epilepsy/ or Seizures/ or seizures, febrile/ or exp status epilepticus/) and (dependen* or dependan*).ti,ab,kf,rx. | 248 |
| | 2 | ((pyridoxine or pyridoxin or pyridoxamine or vitamin b6 or "vitamin b 6") and (dependen* or dependan*) and (epilep* or seizure* or convuls* or spasm*)).ti,ab,kf,rx. | 481 |
| | 3 | Aldehyde Dehydrogenase/df [Deficiency] | 168 |
| | 4 | PDE-ALDH7A1.ti,ab,kf. | 24 |
| | 5 | ((AASA or "α-AASA" or alpha aminoadipic semialdehyde) and dehydrogenase deficien*).ti,ab,kf. | 16 |
| | 6 | ((Antiquitin or ATQ or ASADH) and deficien*).ti,ab,kf. | 58 |
| | 7 | or/1-6 [pyridoxamine dependent epilepsy] | 655 |
| Excluding animal studies | 8 | (exp Animals/ or Models, Animal/ or Disease Models, Animal/) not Humans/ | 5155276 |
| | 9 | 7 not 8 | 593 |
| Limit to English language | 10 | limit 9 to english language | 547 |

### 11.2.2 Retinoblastoma

**Ovid MEDLINE(R) ALL1946 to September 18, 2023**

| **Concept** | **#** | **Search terms** | **Hits** |
|---|---|---|---|
| Retinoblastoma | 1 | Retinoblastoma/ | 8201 |
| | 2 | Retinal Neoplasms/ge | 914 |
| | 3 | Genes, Retinoblastoma/ | 1772 |
| | 4 | (Retina* adj3 (cancer* or tumor* or tumour* or neoplasm* or glioblastoma* or glioma* or neuroblastoma*)).ti,ab,kf. | 1579 |
| | 5 | or/1-4 [retinoblastoma] | 10865 |
| Genetics/ hereditary terms | 6 | Genetics/ | 12916 |
| | 7 | Genetics.fs. | 4008106 |
| | 8 | Genetic disorder/ | 14545 |
| | 9 | exp genetic predisposition to disease/ | 157589 |
| | 10 | Genetic Diseases, Inborn/ | 14545 |

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|---|---|---|---|
| | 11 | (Genetic* or gene or genes or family or families or familial or DNA or hereditary or heritable or heredodegenerative* or inherit* or congenital* or germline or germinal*).ti,ab,kf. | 5087300 |
| | 12 | or/6-11 [Hereditary/ genetics] | 6515297 |
| Retinoblastoma and genetics/ hereditary terms | 13 | 5 and 12 [Retinoblastoma and genetics/ hereditary] | 5395 |
| Excluding animal studies | 13 | (exp Animals/ or exp Models, Animal/ or Disease Models, Animal/) not Humans/ | 5156324 |
| | 14 | 14 not 15 | 5019 |
| Limiting to English language | 15 | limit 15 to english language | 4517 |

### 11.2.3 X-linked hypophosphataemic rickets

**Ovid MEDLINE(R) ALL 1946 to October 03, 2023**

| Concept | # | Search terms | Hits |
|---|---|---|---|
| Hypophosphatemic rickets | 1 | exp Rickets, Hypophosphatemic/ | 952 |
| | 2 | Hypophosphat?emic.ti,ab,kf,rx. | 2433 |
| | 3 | rickets/ or (rickets or rachitides or rachitis).ti,ab,kf,rx. | 10233 |
| | 4 | 2 and 3 | 1644 |
| | 5 | 1 or 4 [Hypophosphatemic rickets] | 2080 |
| Hypophosphatemia | 6 | Hypophosphatemia/ | 1908 |
| | 7 | Hypophosphat?emia*.ti,ab,kf,rx. | 5397 |
| | 8 | or/6-7 [Hypophosphatemia] | 5864 |
| Vitamin d resistant rickets | 9 | (Vitamin d resistant and (rickets or rickets or rachitides or rachitis)).ti,ab,kf,rx. | 608 |
| | 10 | VDRR.ti,ab,kf. | 30 |
| | 11 | or/9-10 [Vitamin d resistant rickets] | 612 |
| Hypophosphatemia or Hypophosphatemic rickets or vitamin d resistant rickets | 12 | 5 or 8 or 11 [Hypophosphatemia or Hypophosphatemic rickets or vitamin d resistant rickets] | 7318 |
| Genetics/ hereditary | 13 | Genetics/ | 12922 |
| | 14 | Genetics.fs. | 4016108 |
| | 15 | Genetic disorder/ | 14548 |
| | 16 | exp genetic predisposition to disease/ | 157711 |
| | 17 | Genetic Diseases, Inborn/ | 14548 |
| | 18 | (Genetic* or gene or genes or family or families or familial or DNA or hereditary or heritable or heredodegenerative* or inherit* or congenital* or germline or germinal*).ti,ab,kf. | 5103147 |
| | 19 | or/13-18 [Genetics/ hereditary] | 6533601 |

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| Hypophosphatemia or Hypophosphatemic rickets and genetics/ hereditary] | 20 | 12 and 19 [Hypophosphatemia or Hypophosphatemic rickets and genetics/ hereditary] | 2327 |
| X-Linked hypophosphataemia or x-linked hypophosphatemic rickets | 21 | ((XLHR or XLH or X Linked) adj3 (hypophosphatemia* or hypophosphataemia*)).ti,ab,kf,rx. | 659 |
| | 22 | (((XLHR or XLH or X Linked) adj3 (Hypophosphatemic or hypophosphataemic)) and (rickets or rachitides or rachitis)).ti,ab,kf,rx. | 580 |
| | 23 | (((XLHR or XLH or X Linked) adj3 vitamin d resistant) and (rickets or rachitides or rachitis)).ti,ab,kf,rx. | 41 |
| Hypophosphatemia or Hypophosphatemic rickets and genetics/ hereditary or x-linked hypophosphataemia/ x linked hypophosphatemic rickets | 24 | or/20-23 | 2656 |
| Excluding animal studies | 25 | (exp Animals/ or exp Models, Animal/ or Disease Models, Animal/) not Humans/ | 5162107 |
| | 26 | 24 not 25 | 2286 |
| Limiting to English language | 27 | limit 26 to english language | 2078 |

### 11.2.4 Familial hemophagocytic lymphohistiocytosis

**Ovid MEDLINE(R) ALL 1946 to September 18, 2023**

| Concept | # | Search terms | Hits |
|---|---|---|---|
| Hemophagocytic Lymphohistiocytosis | 1 | Lymphohistiocytosis, Hemophagocytic/ | 3932 |
| | 2 | ((Hemophagocytic or haemophagocytic or erythrophagocytic) adj3 (lymphohistiocytos* or lymphocytos* or histiocytos* or reticulos* or hymphohistiocytos* or syndrome*)).ti,ab,kf,rx. | 7073 |
| | 3 | or/1-2 [Hemophagocytic Lymphohistiocytosis] | 7531 |
| Genetics/ hereditary terms | 4 | Genetics/ | 12920 |
| | 5 | Genetics.fs. | 4010483 |
| | 6 | Genetic disorder/ | 14545 |
| | 7 | exp genetic predisposition to disease/ | 157614 |
| | 8 | Genetic Diseases, Inborn/ | 14545 |
| | 9 | (Genetic* or gene or genes or familial or family or families or DNA or hereditary or heritable or heredodegenerative* or inherit* or congenital* or germline or germinal*).ti,ab,kf. | 5090788 |
| | 10 | or/4-9 [Genetics/ hereditary] | 6519551 |

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|---|---|---|---|
| Hemophagocytic Lymphohistiocytosis and genetics/ hereditary | 11 | 3 and 10 | 2608 |
| Hemophagocytic Lymphohistiocytosis and genetics/ hereditary or phrase searches for condition | 12 | ((Primary adj4 (hemophagocytic or haemophagocytic or erythrophagocytic)) and (lymphohistiocytosis or lymphocytos* or histiocytos* or reticulos* or hymphohistiocytos* or syndrome*)).ti,ab,kf,rx. | 208 |
| | 13 | (FHLH or PHLH).ti,ab,kf. | 98 |
| | 14 | or/11-13 | 2699 |
| Excluding animal studies | 15 | (exp Animals/ or exp Models, Animal/ or Disease Models, Animal/) not Humans/ | 5158092 |
| | 16 | 14 not 15 | 2647 |
| Limit to English language | 17 | limit 16 to english language | 2386 |

### 11.2.5 Medium-chain acyl-CoA dehydrogenase deficiency

**Ovid MEDLINE(R) ALL 1946 to September 18, 2023**

| Concept | # | Search terms | Hits |
|---|---|---|---|
| MCADD | 1 | Acyl-CoA Dehydrogenase/ and deficien*.ti,ab,kf,rx. | 658 |
| | 2 | Acyl-CoA Dehydrogenase/df, ge, me | 550 |
| | 3 | MCADD.ti,ab,kf. | 140 |
| | 4 | ((MCAD or MCADH or MCACA) and deficien*).ti,ab,kf. | 368 |
| | 5 | (medium-chain acyl-CoA dehydrogenase adj2 deficien*).ti,ab,kf,rx. | 568 |
| | 6 | (medium-chain acyl-coenzyme A dehydrogenase adj2 deficien*).ti,ab,kf. | 87 |
| | 7 | (medium chain acyl dehydrogenase adj2 deficien*).ti,ab,kf. | 0 |
| | 8 | MCACA dehydrogenase deficien*.tw,kw. | 0 |
| | 9 | Octanoyl-CoA dehydrogenase deficien*.ti,ab,kf. | 0 |
| | 10 | Octanoyl-coenzyme A dehydrogenase deficien*.ti,ab,kf. | 0 |
| | 11 | or/1-10 [MCADD] | 1147 |
| Excluding animal studies | 12 | (exp Animals/ or exp Models, Animal/ or Disease Models, Animal/) not Human/ | 5158092 |
| | 13 | 11 not 12 | 986 |
| Limit to English lanaguage | 14 | limit 13 to english language | 931 |