



# Rapid exome sequencing: revolutionises the management of acutely unwell neonates

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## Abstract

Diagnosing acutely unwell infants with a potential genetic diagnosis can be challenging for healthcare professionals. Evidence suggests that up to 13% of critically unwell infants on the neonatal intensive care unit (NICU) have an underlying molecular diagnosis and when identified directly affects treatment decisions in 83%. On 1st October 2019, the National Health Service England (NHSE) launched a nationally commissioned service so that rapid whole-exome sequencing can be offered to critically unwell babies and children with a likely monogenic disorder who are admitted to NICU and paediatric intensive care unit (PICU). We present 7 cases from two neonatal units in the West Midlands (UK), where rapid exome sequencing has revealed a genetic diagnosis. Early genetic diagnosis in this cohort has influenced management in all (100%) cases, and in 57% (4 in 7 cases), it has helped in the decision to reorientate care. In some cases, early diagnosis has reduced the need for invasive and unnecessary investigations and avoided the need for post-mortem investigations. The genetic diagnosis has helped in counselling the families regarding the recurrence risk for future pregnancies. In some cases, this has provided parents with the reassurance of a low recurrence. In others, it has resulted in the offer of prenatal diagnosis or assisted conception technologies.

## What is Known:

- Rapid whole-exome sequencing was commissioned in the UK in October 2019.
- It is available for critically unwell babies with a likely monogenic aetiology.

## What is New:

- It helps management planning for rare genetic disorders and future pregnancies counselling.
- It can reduce the need for invasive investigations and overall intensive care costs.

**Keywords** Neonatal intensive care · Genetics · Rapid whole-exome sequencing

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## Introduction

Genetic disorders are a leading cause of neonatal morbidity and mortality. West Midlands (UK) has an increased rate of recessive disorders due to the high incidence of consanguineous marriages, accounting for a larger number of the neonatal admissions. Despite extensive investigations, a definitive diagnosis is not always found, leading to difficulties in predicting prognosis and future care planning.

Over the recent years, whole-exome sequencing has become increasingly available, having the maximum benefit when applied early in life [1]. A rapid molecular genetic diagnosis in critically unwell infants can reduce the need for invasive and often unnecessary diagnostic investigations and direct future multidisciplinary care planning [2–4]. Evidence

suggests that up to 13% of critically unwell infants on the neonatal intensive care unit (NICU) have an underlying molecular diagnosis and when identified directly affect treatment decisions in 83% [1].

On 1st October 2019, the National Health Service England (NHSE) launched a centrally funded national service through the Exeter Genomics Laboratory so that rapid whole-exome sequencing can be offered to acutely unwell babies and children with a likely monogenic aetiology who are admitted to the NICU and paediatric intensive care unit (PICU). The service is offered as R14 clinical indication in the new National Genomic Test Directory. The main aim of this service is to provide rapid genetic diagnosis which can influence immediate management decisions [5]. This consists of trio (affected child and both unaffected parents) exome sequence analysis of the coding region and conserved splice sites of 23,244 genes [5]. The analysis requires genomic DNA extracted from peripheral blood samples. The DNA is prepared for sequencing as per the manufacturer's protocol (Twist Biosciences) and sequenced on a NextSeq 500 or NovaSeq sequencer (Illumina, San Diego, CA, USA). The data is processed following GATK (v3.4) best practice guidelines [6]. An in-house bioinformatics pipeline is applied to identify *de novo*, compound heterozygous, homozygous or X-linked variants. Copy number variants are identified using read depth analysis with an in-house tool and comparing the test sample against reference samples. The variants identified are classified according to the ACMG-AMP guidelines for variant interpretation [7]. Microarray and other relevant genetic testing are requested in parallel to the exome sequencing, as a diagnosis is required urgently in acutely unwell infants. In addition, the exome sequencing pipeline in the Exeter Genomics Laboratory is not validated to detect copy number variations. The genomic data is interpreted by a clinical scientist, and the results are then discussed with the clinical team.

Between October 2019 and September 2020, 8 cases were referred for rapid exome sequencing from two neonatal units in the West Midlands (UK). All cases were infants admitted to the NICU with an unclear underlying diagnosis and suspected to have a monogenic aetiology following multidisciplinary team (MDT) evaluation. Rapid exome sequencing revealed a genetic diagnosis in 7 cases, which in turn has influenced management decisions (Table 1).

## Discussion

Prior to the introduction of whole-exome sequencing, despite extensive genetic investigations, it was not always possible to establish a rare genetic diagnosis. This can lead to diagnostic uncertainty amongst the multidisciplinary team and difficulties in predicting prognosis and coordinating future care planning. It can also significantly affect parental well-being and

can cause parental uncertainty. Each of these seven cases demonstrates the benefits of being able to obtain a rapid molecular diagnosis in critically unwell neonates.

The mean turnout time for the rapid exome result was around 11 days, and in each case, the diagnosis was received within 4 weeks of life allowing for early care planning and limiting unnecessary invasive investigations such as muscle biopsy. Becoming more familiar with this technology allows clinicians to use it as primary investigation, reducing the turnaround time, which can help in the early management of the neonates.

## Futility of continued intensive care

A decision to re-orientate care for acutely unwell babies can be extremely challenging especially when the underlying diagnosis is not known.

In cases 1, 5, 6 and 7, early diagnosis confirmed the futility of continued intensive care, parents and health professionals agreeing palliative care was in the baby's best interests.

X-linked myotubular myopathy (case 1) occurs in 1 in 50,000 live male births, causing progressive myopathy and hypotonia. Approximately 80% of affected males present with severe (classic) X-MTM characterised by polyhydramnios, decreased foetal movement and neonatal weakness and hypotonia. Respiratory failure is nearly uniform, with most individuals requiring 24-h ventilatory assistance [8]. In this case, exome sequencing detected a nonsense variant which is associated with the severe spectrum of the disorder. This genotype phenotype correlation was helpful to counsel the parents regarding the poor prognosis; the baby was ventilator-dependent at day 25, and hence, a decision of reorientation of care was discussed. The muscle biopsy result was available 6 days later and was consistent with the genetic diagnosis.

For infants born with multiple congenital anomalies (case 7), various investigations may clearly point towards extremely poor prognosis. However, without a definitive diagnosis, it can be challenging to counsel parents. This may lead to unnecessary delay in the initiation of palliative care. A definitive genetic diagnosis can help health professionals to confidently counsel parents, and it also aids parents to come to terms with the clinical outcome.

## Multidisciplinary care planning

Obtaining an early diagnosis for cases 2 and 3 facilitated early multidisciplinary medical and surgical care planning prior to discharge from the NICU.

X-linked Ohdo syndrome (case 2) is a rare syndrome occurring in less than one per million live births characterised by multi-system anomalies, dysmorphic features, delayed

**Table 1** Rapid whole-exome sequencing outcomes and impact on care

Case	Phenotype	Genetic diagnosis	Gene	Variant details	Turnaround time (DNA receipt in testing lab to reporting time)	Timing of diagnosis (day of life)	Impact on proband's care	Implications for the family
1	Respiratory failure, hypotonia, long fingers and toes and cryptorchidism	X-linked myotubular myopathy	<i>MTM1</i>	Hemizygous maternally inherited (NM_000252.2:c.1381C>T p.(Gln461Ter)	11 days	25	Reorientation of care. (withdrawal of respiratory support)	X-linked inheritance (50% recurrence risk for male babies) Prenatal including foetal sexing/PGD options discussed. Extended family screening offered
2	Dysmorphic features, hiatus hernia, tetralogy of Fallot, cryptorchidism	X-linked Ohdo syndrome	<i>MED12</i>	<i>De novo</i> hemizygous (NM_005120.2:c.4831C>T p.(Arg1611Cys))	10 days	22	Surgical and medical management planning	<i>De novo</i> Likely Low recurrence risk
3	Dysmorphic features, flickering eyelids, poor feeding	WOREE syndrome	<i>WWOX</i>	Homozygous deletion arr[hg19]16q23.1(77745748_78187074)x0 Parents, heterozygous carriers	10 days	24	MDT community care planning	Autosomal recessive (25% recurrence risk) Prenatal/PGD options discussed Extended family screening offered
4	Severe hydrops fetalis	Noonan syndrome	<i>PTPN11</i>	<i>De novo</i> heterozygous (NM_002834.4:c.1471C>T, p.(Pro491Ser))	9 days	17	Screening for associated congenital anomalies	<i>De novo</i> Likely low recurrence risk
5	Respiratory failure, hypertonica, seizures	Asparagine synthetase deficiency	<i>ASNS</i>	Homozygous (NM_133436.3:c.1286_1289del p.(Tyr429Cysfs*7)) Parents, heterozygous carriers	7 days	15	Reorientation of care (withdrawal of respiratory support)	Autosomal recessive (25% recurrence risk) Prenatal/PGD options discussed Extended family screening offered
6	Respiratory failure, hypotonia, skeletal abnormalities	Nemaline myopathy, type 10	<i>LAMOD3</i>	Homozygous (NM_198271.4:c.882dup p.(Asp295Argfs*2)) Parents, heterozygous carriers	11 days	23	Reorientation of care (withdrawal of respiratory support)	Autosomal recessive (25% recurrence risk) Prenatal/PGD options discussed
7	Complex congenital brain abnormalities (lissencephaly, ventriculomegaly, pontocerebellar hypoplasia), congenital airway abnormalities (laryngomalacia, micrognathia, cleft palate)	TUBB2B-related complex cortical dysplasia with other brain malformations	<i>TUBB2B</i>	<i>De novo</i> heterozygous (NM_178012.4:c.1184T>C.p.(Leu395-Pro))	8 days	24 days	Reorientation of care (withdrawal of respiratory support)	<i>De novo</i> Likely low recurrence risk

development and learning difficulties [9]. This rare diagnosis had implications on genetic counselling related to the long-term prognosis.

WOREE syndrome (case 3) is a severe epileptic encephalopathy characterised by the absence of language development and acquisition of walking, early-onset drug-resistant seizures, ophthalmological involvement and a high likelihood of premature death [10]. The genetic diagnosis was made by microarray analysis and also by exome sequencing. Obtaining a diagnosis meant parents could be counselled about long-term prognosis. Early involvement of the palliative care team ensured there was an advanced care plan in place to support parents in their home environment.

In these extremely rare conditions, increasing genetic identification can improve our medical knowledge about the expected outcome and prognosis, which will be helpful in effective counselling of families.

## Medical investigations

Hydrops fetalis is the abnormal accumulation of fluid in at least two different foetal compartments. Non-immune hydrops fetalis is a complex condition with numerous underlying causes, estimated to affect 1 in 3000 pregnancies [11]. The early diagnosis of Noonan syndrome in case 4 avoided a number of unnecessary and potentially invasive investigations to find out the cause of hydrops. The diagnosis was helpful in screening for known associations commonly seen in Noonan syndrome, such as congenital heart abnormalities.

## Pregnancy counselling and preimplantation diagnosis

Asparagine synthetase deficiency (case 5) is a rare autosomal recessive neurological disorder; only 22 cases have been reported in the literature [12]. It is characterised by microcephaly, initial axial hypotonia followed by evolving spastic quadriplegia, severe developmental delay, cortical atrophy and seizures [12, 13]. Half of infants die by 1 year of age [12]. The recurrence risk for a future pregnancy is 25%. Early diagnosis and genetic counselling can support families through pregnancy planning for not only the parents and also for the extended family members in multiple consanguineous families.

## Health economics

Early diagnosis and management planning can reduce the cost of expensive neonatal intensive care which can be up to £2000 per day, as well as precious tertiary NICU cot availability for

most needed infants. This will have significant impact on long-term health economics. A confirmed genetic diagnosis can avoid expensive post-mortem examination. Consenting for a baby's post-mortem can be very traumatic experience for parents and can be avoided.

Early genetic diagnosis has resulted in the availability of the prenatal diagnosis or assisted conception technologies to enable families the possibility of making informed reproductive choices and avoiding the distress of having a child with a potentially lethal condition; the cost and benefits of these are immeasurable.

## Conclusion

The aim of this NHS service is to provide genetic testing for infants and children who are acutely unwell, a service that previously was not available in the UK. All of the above diagnoses would have been found by a conventional genetic approach, taking months to years to establish a diagnosis; however in these cases, the infants were acutely unwell, and rapid diagnosis was essential.

We demonstrated the clinical utility of this service. Efforts have been directed at MDT collaboration and continued education and training to embed and integrate rapid exome sequencing into routine clinical practice in the NHS, benefiting acutely unwell infants and their extended families.

**Abbreviations** *NHSE*, National Health Service England; *NICU*, Neonatal intensive Care Unit; *PICU*, Paediatric intensive care unit

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## Declarations

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**Consent for publication** N/A.

**Conflict of interest** The authors declare no competing interests.

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