Mortality in a neonate with molybdenum cofactor deficiency illustrates the need for a comprehensive rapid precision medicine system

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Abstract Neonatal encephalopathy with seizures is a presentation in which rapid whole-genome sequencing (rWGS) has shown clinical utility and improved outcomes. We report a neonate who presented on the third day of life with seizures refractory to antiepileptic medications and neurologic and computerized tomographic findings consistent with severe generalized brain swelling. rWGS revealed compound heterozygous variants in the molybdenum cofactor synthesis gene, type 1A (MOCS1 c.*7 + 5G > A and c.377G > A); a provisional diagnosis of molybdenum cofactor deficiency on day of life 4. An emergency investigational new drug application for intravenous replacement of the MOCS1 product, cyclic pyranopterin monophosphate, was considered, but felt unsuitable in light of the severity of disease and delay in the start of treatment. The patient died on day of life 9 despite having a precise molecular diagnosis within the first week of life. This case illustrates that an rWGS-based molecular diagnosis within the first week of life may be insufficient to improve outcomes. However, it did inform clinical decision-making with regard to resuscitation and predicted long-term outcome. We suggest that to achieve optimal reductions in morbidity and mortality, rWGS must be implemented within a comprehensive rapid precision medicine system (CRPM). Akin to newborn screening (NBS), CRPM will have onboard-diagnosis, and precision medicine implementation components developed in response to patient and parental needs. Education of health-care providers in a learning model in which ongoing data analyses informs system improvement will be essential for optimal effectiveness of CRPM.

INTRODUCTION

Genetic diseases are very common among infants in intensive care units (ICUs). A recent study reported an incidence of 16% (Kingsmore et al. 2019). Genetic diseases are a leading cause of morbidity and mortality among infants in ICUs (Berry et al. 2008; Weiner et al. 2011; March of Dimes 2016; Murphy et al. 2018). Disease progression can be extremely rapid in neonates, necessitating early etiologic diagnosis and initiation of effective therapies to lessen suffering, morbidity, and mortality (Petrikin et al. 2015; Smith et al. 2015). Timely
diagnosis of genetic diseases requires genome-wide testing because more than 14,000 simple genetic diseases have been described and their presentations often overlap in newborns (Database of Human Structural Variants 2019; Online Mendelian Inheritance in Man 2019). Rapid whole-genome sequencing (rWGS) has demonstrated diagnostic and clinical utility in seriously ill newborns with diseases of unknown etiology in ICUs (Willig et al. 2015; Famaes et al. 2018; Mestek-Boukhibar et al. 2018; Petrikin et al. 2018; French et al. 2019). By providing a molecular diagnosis in the first days of life, rWGS can enable timely institution of genome-informed medicine that can transform outcomes. Likewise, the absence of likely pathogenic variants in specific genes significantly reduces the likelihood of genetic disorders causing the current presentation, modifying the differential diagnosis and informing decisions regarding the appropriateness of complex interventions. Over the last 8 years, methods have been developed for increasingly rapid diagnosis of genetic diseases by rWGS, with a major focus on improved analytic and diagnostic performance (Saunders et al. 2012; Miller et al. 2015; Clark et al. 2019). Here we present a case that illustrates the need for rWGS to be implemented in routine practice within a comprehensive rapid precision medicine (CRPM) system to optimize outcomes.

**CASE PRESENTATION**

The patient was a Latino male infant born at 40 wk gestation via repeat cesarean section. Apgar scores were 9 at 1 and 5 min. He weighed 3.6 kg at birth (53rd percentile), with a head circumference of 34.5 cm (35th percentile) and length of 53.3 cm (79th percentile). He was discharged home after an uneventful 48-h nursery stay. Family history was significant for an older sibling who had died in Mexico at 3 days of life (DOL). The listed cause of death was sudden infant death syndrome.

Rhythmic jerking of the infant’s arms and eye-rolling began on DOL 3. At a local Emergency Department, he was noted to have spastic movements of both arms and his gaze was fixed, with horizontal nystagmus. He was transferred by helicopter to Rady Children’s Hospital level IV neonatal ICU. At admission, he was irritable and his arms were pronated and flexed. He had an absent suck reflex and bilateral downward eye deviation. No dysmorphic features were noted (Table 1). Initial laboratory values were within normal ranges. Computerized axial tomography of the head showed diffuse loss of gray/white matter junctions and indistinctness of cortical sulci, gyri, and boundaries of basal ganglia. Ventricles were slit-like and the Sylvian fissure was not well-delineated. Brain magnetic resonance imaging revealed severe generalized brain swelling (edema) with restricted cortical diffusion. The electroencephalogram demonstrated abundant seizures. The seizures were refractory to antiepileptic medications.

Following receipt of verbal consent, rWGS was performed on the proband and his mother on the day of admission (DOL 3). The proband genome coverage was 52-fold and 98.2% of variants had quality scores >40. The proband genome had no regions of homozygosity of >5 Mb. A low serum uric acid (<0.5 mg/dL, reference range 2.0–7.0 mg/dL) was reported on DOL 4. This suggested molybdenum cofactor deficiency (MOCOD), caused by biallelic pathogenic variants in either of the molybdenum cofactor synthesis genes MOCS1 or MOCS2 or in Gephyrin (GPHN) were high on the differential diagnosis. Two likely pathogenic variants (c.∗7 + 5G > A and c.377G > A) were identified in MOCS1 (Table 2), which encodes two enzymes, MOCS1A and MOCS1B, in a single transcript. MOCOD is a rare inherited metabolic disorder resulting in a combined deficiency of aldehyde oxidase (EC 1.2.3.1), xanthine dehydrogenase (EC 1.1.1.204), and sulfite oxidase (EC 1.8.3.1). Most patients with MOCOD present soon after birth with intractable seizures and undetectable serum uric acid (Arenas et al. 2009). MOCS1 c.∗7 + 5G > A was a splice region variant, reported in the heterozygous
Table 1. Observed and expected phenotypic features

| Expected phenotypes for MOCOD (OMIM ID #252150) | Observed phenotypes |
|-------------------------------------------------|---------------------|
| Poor growth                                     | Absent              |
| Frontal bossing                                 | Absent              |
| Microcephaly                                    | Absent              |
| Macrocephaly                                    | Absent              |
| Long face                                       | Absent              |
| Puffy cheeks                                    | Absent              |
| Long philtrum                                   | Absent              |
| Dislocated lenses                               | Absent              |
| Spherophakia                                    | Absent              |
| Nystagmus                                       | Present             |
| Elongated palpebral fissures                    | Absent              |
| Widely spaced eyes                              | Absent              |
| Small nose                                      | Absent              |
| Thick lips                                      | Absent              |
| Poor feeding                                    | Present             |
| Asymmetric skull                                | Absent              |
| Myoclonic spasms                               | Present             |
| Absent psychomotor development                 | n.a.                |
| Intractable seizures, intractable               | Present             |
| Opiosthotonos                                   | Absent              |
| Hypertonicity                                   | Present             |
| Spastic quadriplegia                            | Absent              |
| Cerebral atrophy                                | Absent              |
| Thinning of the corpus callosum                 | Absent              |
| Gliosis                                         | Absent              |
| Demyelination                                   | Absent              |
| Axonal loss                                      | Absent              |
| Cystic lysis of the deep white matter           | Absent              |
| Enlarged ventricles                             | Absent              |
| Hypouricemia                                    | Present             |
| Increased urinary xanthine                      | n.d.                |
| Increased urinary hypoxanthine                  | n.d.                |
| Increased urinary S-sulfocysteine               | Present             |
| Increased urinary taurine                       | n.d.                |
| Xanthine stones                                 | Absent              |
| Decreased xanthine dehydrogenase activity       | n.d.                |
| Decreased sulfite oxidase activity              | n.d.                |
| Molybdenum cofactor deficiency                  | n.d.                |
| Onset at birth                                  | Present             |
| Progressive disorder                            | Present             |
| Death in childhood                              | Present             |

(MOCOD) Molybdenum cofactor deficiency, (n.a.) not applicable, (n.d.) not determined.
state in six of 246,238 gnomAD chromosomes (PM2). This variant was predicted to reduce splicing at the 5′ donor site of intron nine. Homozygous substitution of the neighboring nucleotide (c.∗7 + 6T > C) in a patient with MOCOD was associated with abnormal splicing predicted to result in absence of MOCS1A (Arenas et al. 2009; Hinderhofer et al. 2017). c.∗7 + 5G > A was initially scored as a variant of uncertain significance, but after consultation with the clinical team and in light of low serum uric acid, the variant was upgraded to likely pathogenic on the basis of PM1 (proximity to c.∗7 + 6T > C, for which functional studies showed aberrant splicing [Arenas et al. 2009], together with high level of conservation and similar splice effects predicted in c.∗7 + 6T > C), PM2, PP3 (NNSplice v0.9: −0.575 [predicted to weaken the more 3′ donor site at c.∗7 + 1], GeneSplicer = −1.987 [predicted to weaken the more 3′ donor site at c.∗7 + 1], MaxEntScan = −9.282 [predicted to weaken the more 3′ donor site at c.∗7 + 1]), and PP4 (Richards et al. 2015). MOCS1 c.377G > A has been previously reported in a patient with MOCOD (Arenas et al. 2009; Hinderhofer et al. 2017) (PM3) and was also rare (heterozygous in 14 of 245,472 gnomAD chromosomes, PM2). It was scored as likely pathogenic on the basis of PM3 (in trans with c.418 + 1G > A in Hinderhofer et al. 2017), PM2, PP3 (Mutation Taster: 1, PolyPhen-2—HDIV (v2.2.2): 0.997, SIFT: 0), and PP4 (Richards et al. 2015). No other diagnostic variants were found in other genes. A provisional diagnosis of MOCOD was reported to the NICU team in 34 h (DOL 4). The diagnosis was confirmed on DOL 9 by elevated urinary S-sulfocysteine (731 µmol/g creatinine, reference range ≤80 µmol/g creatinine; ordered on DOL 3).

MOCS1 catalyzes the production of cyclic pyranopterin monophosphate (cPMP), the first step in the synthesis of molybdenum cofactor (Schwahn et al. 2015). Daily intravenous replacement of cPMP restores molybdenum cofactor–dependent enzyme activities (Schwahn et al. 2015). If replacement is started before onset of encephalopathy, long-term developmental outcomes are good (Schwahn et al. 2015). Subsequent treatment may alleviate symptoms. Unfortunately, 15 years after the first infant with MOCOD benefitted from cPMP (Schwarz et al. 2004), and despite the FDA “breakthrough therapy” designation in 2013, cPMP remains an experimental therapy and no clinical trials are currently enrolling neonates. Commercial rights to cPMP have been transferred at least three times, most recently to Origin Biosciences in 2016. A single-patient Emergency Investigational New Drug (eIND) application was considered in our neonate. However, in light of the severity of disease and anticipated delay of days to weeks in commencing treatment, he was not considered a suitable candidate. On DOL 9, he became apneic and severely bradycardic. Despite aggressive resuscitation, he continued to decline clinically, developing severe lactic acidosis in the setting of poor cardiac function and respiratory failure. Care was redirected and the patient died of his underlying disease.

**DISCUSSION**

MOCOD is a prototypic example of a very rare genetic disorder whose outcome may be changed by prompt diagnosis and treatment. The onset of symptoms in MOCOD occurs
typically on the first day of life with seizures (Mechler et al. 2015). In the absence of rapid genomic sequencing, the median age at diagnosis is 4.5 mo (Mechler et al. 2015). Here, diagnosis was made on the fourth day of life, a day after clinical and imaging evidence of severe brain damage. As a result, the decision was made not to proceed with cPMP therapy. Regrettably, the infant’s sibling likely died of the same condition. Had a molecular diagnosis been made in that sibling, fetal or perinatal diagnosis would have been possible in the proband, enabling early delivery by cesarean section, replacement of cPMP at birth, and early institution of supportive care. MOCOD is frequently misdiagnosed as hypoxic–ischemic encephalopathy or cerebral palsy (Topcu et al. 2001; Kikuchi et al. 2012; Zahid et al. 2019).

This case powerfully illustrates rWGS that provides a molecular diagnosis within the first week of life may be insufficient to improve outcomes. In neonatal encephalopathy, even if rWGS is ordered promptly and performed rapidly in the context of a regional ICU with a full complement of pediatric subspecialists experienced in newborn genomic medicine, a molecular diagnosis may be made too late to improve outcomes. To decrease newborn morbidity and mortality optimally, we suggest that rWGS must be implemented within a comprehensive system for rapid delivery of precision medicine (CRPM) (Stark et al. 2018; Pearce et al. 2019). As rWGS gains broader use in infants in ICUs worldwide, such a CRPM system will be particularly needed in hospitals lacking the full complement of pediatric subspecialists, including neurologists or medical geneticists experienced in CRPM.

National systems of newborn screening (NBS) of healthy neonates for 30 to 50 genetic disorders that have very early progression and effective treatments have reduced their morbidity and mortality (Berry 2015; Therrell et al. 2015). Upon approval of cPMP, MOCOD may be suitable for inclusion in genomic-based NBS or expanded carrier screening programs. The pretest probability of genetic diseases in seriously ill neonates in ICUs with diseases of unknown etiology is about 10,000-fold greater than in healthy infants receiving NBS (Kingsmore et al. 2019). Nevertheless, the components of an effective CRPM system would be rather similar to those of NBS (Fig. 1; Therrell et al. 2010), including the following.

1. **Iterative development and administration of payer-specific, state and national policies** are needed to (a) define indications for rWGS; (b) evaluate the robustness of evidence of efficacy for individual genetic disease treatments; (c) establish quality metrics to improve CRPM performance; and (d) require insurance authorization for reimbursement. Because such policies will be developed incrementally over several years for thousands of conditions there is an interim need for pioneering regional hospitals to establish centers of excellence in CRPM. This was the rationale behind the Vermont Oxford Rady Children’s Genomic Network (https://publicvtoxfordorg/genomicnetwork).

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**Figure 1.** Components of the proposed comprehensive system for delivery of newborn precision medicine.
2. **Ascertainment** encompasses (1) rapid ascertainment of newborns who may benefit from CRPM; (2) education and equipping of providers to obtain informed consent and explain scope of interpretation; (3) creation of rWGS order sets in electronic health records (EHRs); (4) rapid authorization of rWGS; (5) rapid blood sample collection and shipment; (6) communication of sufficient details of the phenotype and near-term clinical decision-making to facilitate diagnosis with appropriate time to result. Hitherto, rWGS-based CRPM has largely been limited to research studies with clear inclusion/exclusion criteria and dedicated program managers and enrollment and genetic counseling personnel. Ascertainment activities require iterative development, provider education, and onboarding. In the current case, ascertainment was seamless. However, in general we have found the switch from funded research rWGS to prior authorization of commercial testing to be not straightforward and that it required peer-to-peer communication and education of providers and payers. Ascertainment activities must be specific, contextually sensitive, semiautomated, and integrated into the EHRs. For example, we developed an rWGS ordering portal that was linked to the EHRs and interpretation software portal to facilitate ascertainment activities (Clark et al. 2019). We have also implemented quality assessment to measure the proportion of suitable infants in whom rWGS is performed, time to test order, and rate of prior authorization.

3. **Early diagnosis** encompasses sample receipt through reporting of preliminary and final results. In the current case, a provisional diagnosis of MOCOD was reported in 34 h. Although time from sample receipt to provision of preliminary results can be as short as 19 h (Clark et al. 2019), further automation is needed to achieve this in routine clinical practice. Standards for analytic and diagnostic performance and variant interpretation of genomic sequencing reflect consequences for gene function but do not currently consider the clinical context (Rehm et al. 2013; Aziz et al. 2015; Richards et al. 2015; Matthijs et al. 2016; Roy et al. 2018). In the context of acutely ill infants in ICUs with diseases of unknown etiology, the pretest probability of an underpinning genetic disease is high and, absent CRPM, morbidity and mortality are extreme, as exemplified in the current case. Specific, contextually congruous standards are needed for classification of likelihood of molecular diagnosis in this population. For acutely ill infants in ICUs, the goodness of fit of observed phenotypes to those of newborn diseases suggested by genomic variants, presence of pathognomonic features, availability of timely confirmatory diagnostic studies, and the risk/benefit ratio of immediate treatment for the associated disorder should also be important determinants of likelihood of a molecular diagnosis. An example has been proposed by the European Society for Human Genetics (https://wwweshgorg/fileadmin/wwweshgorg/documents/Variant_classification_system/Variant_class_ESHGpdf). In the current case, the classification of the pathogenicity of MOCS1 c.7 + 5G > A was not straightforward. For optimal effectiveness in NICUs, rWGS reports must be communicated in a manner that is comprehensible to ICU teams. Effective communication requires some molecular genetic education of NICU teams, deobfuscation of verbal and written reports, and individualized guidance about certainty of specific diagnoses (akin to ACT sheets in NBS) (ACMG ACT sheets 2019). Finally, the diagnostic phase should be subject to a quality improvement cycle to ensure continuous enhancement of analytic and diagnostic performance, including the time to reporting and effectiveness of communication of results.

4. **Precision medicine** encompasses clear communication to parents, genetic counseling, and both immediate and lifelong, genome-informed modification of treatment. Although there are more than 14,000 genetic diseases, only 725 currently have published management guidelines (Adam et al. 2019). As in the current case, treatments may not be FDA-approved. Many genetic disease treatments have either not been subject to clinical
trials or evidence of efficacy and effectiveness is limited to case reports or small case series. Furthermore, the prognosis and complications of many genetic diseases are not well understood or are highly variable. Thus, there exists an immense need to integrate evidence of therapeutic efficacy and to provide high-quality clinical decision support to ICU teams for rare genetic diseases. Where treatments are not readily available, as herein, there exists the need for CRPM to include support services, such as expert navigation and preparation of eIND applications and reports (https://www.fda.gov/drugs/ investigational-new-drug-ind-application/emergency-ind-timeline). Not infrequently, newborns will have been discharged before the return of results. Because neonatology is an inpatient specialty, the postanalytic components, as with NBS, must routinely include follow-up, frequently for medically complex patients. Akin to NBS, treatment is often lifelong. Finally, as with the other components, there exist substantial needs both to educate health-care providers and to assess quality, with goals related to parental understanding of results, their satisfaction with care, clinical utility of results, and long-term outcomes.

Finally, the data generated for each case should be collected in a knowledgebase for ongoing system performance assessment and revision of NBM policy. In time, as individual genetic diseases are recurrently diagnosed, that knowledgebase will become a powerful resource for improvement of interpretation, genetic counseling, and treatment (Chambers et al. 2016).

**METHODS**

Following receipt of verbal consent, rWGS was performed on the proband and his mother on the day of admission at our California-licensed, Clinical Laboratory Improvement Amendments (CLIA)-certified and College of American pathologists (CAP)-accredited laboratory as previously described (Clark et al. 2019). Following DNA extraction from whole blood, sequencing libraries were constructed using the TruSeqDNA PCR-Free Library Prep kit (Illumina) according to the manufacturer’s instructions. Paired-end sequencing was performed on a NovaSeq 6000 and S1 flowcell (Illumina) (Table 3). The DRAGEN processor (Illumina) was used for rapid alignment and nucleotide variant calling (Table 3). The proband genome had no regions of homozygosity of >5 Mb. Variant analysis and interpretation were performed using the Clinical Reporter (Fabric Genomics, version 6.4.1). Variants affecting splicing were predicted by MaxEntScan, NNSPLICE, and GeneSplicer (Reese et al. 1997; Pertea et al. 2001; Yeo and Burge 2004). Human Phenotype Ontology terms used at interpretation were seizures (HP:0001250) and lactic acidosis (HP:0003128). Variants were filtered to retain those with allele frequencies of <0.5% in population databases, and classified

| Table 3. Proband genome sequencing metrics |
|---|---|
| Metric | Value |
| Read length | 2 × 100 nt |
| Mean coverage | 52-fold |
| Nucleotide variants identified | 4,829,581 |
| Variants with quality scores >40 | 98.2% |
| Coding nucleotide variants identified and | 26,202 |
| Homozygous: heterozygous ratio of coding nucleotide variants | 0.59 |
| Transition to transversion ratio of coding nucleotide variants | 2.88 |
according to American College of Medical Genetics and Genomics (ACMG)/Association of Molecular Pathology (AMP) guidelines. The likely causative variants were orthogonally confirmed by PCR and Sanger sequencing.

ADDITIONAL INFORMATION

Data Deposition and Access
Sequencing data is not publicly available because this was a quality improvement project with Institutional Review Board waiver. Thus, informed written consent to share sequencing data was not obtained. The ClinVar (https://www.ncbi.nlm.nih.gov/clinvar/) accession numbers for c.*7+5G>A and c.377G>A are VCV000692068.1 and VCV000692063.1, respectively.

Ethics Statement
Clinical rWGS was performed as part of Project Baby Bear, a Medi-Cal quality improvement project funded by the California Department of Health Care Services. Verbal consent for mother–infant duo rWGS was obtained. The Institutional Review Board of Rady Children’s Hospital, San Diego, and University of California–San Diego issued a waiver for Project Baby Bear.

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Author Contributions
S.F.K. wrote the manuscript. All authors reviewed the final version. N.R., A.F., K.J., and A.-K.N. performed chart review and wrote the clinical presentation. K.J., S.N., and S.C. performed variant interpretation. Y.D. supervised laboratory operations. D.D. provided genomic medicine guidance. W.B., C.H., D.D., and S.F.K. developed the strategy for CRPM.

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