Journal of Paediatrics and Child Health

doi:10.1111/jpc.15955

VIEWPOINT

Neonatal Bartter syndrome diagnosed by rapid genomics following low risk pre-conception carrier screening

Thomas A Forbes [1,2,3] Jane Wallace,[4] Smitha Kumble,[4] Martin B Delatycki[2,4] and Zornitza Stark[2,4,5]

[1]Department of Nephrology, Royal Children's Hospital, [2]Department of Paediatrics, University of Melbourne, [3]Kidney Regeneration Group, [4]Victorian Clinical Genetics Services, Murdoch Children's Research Institute and [5]Australian Genomics Health Alliance, Melbourne, Victoria, Australia

Advances in the speed and accessibility of genomic sequencing are broadening the application of this technology to rapid, acute care diagnostics and pre-conception carrier screening. In both circumstances, genetic counselling plays a critical role in preparing couples for the strengths and limitations of the testing. For pre-conception carrier screening in particular, it is important that parents and clinicians are aware that even in the absence of an identified risk for recessive disease, a baby with a genetic condition may still be conceived. As an example, we present the genomic journey of a couple who underwent pre-conception carrier screening and following a low-risk result, delivered a baby boy who was diagnosed with Type 1 Bartter syndrome. Ultra-rapid, post-natal, trio whole genome sequencing resolved both parents as carriers of pathogenic variants in *SLC12A1*, a gene not included in the original pre-conception screening panel. This family's story highlights (i) the intricacy of gene selection for pre-conception screening panels, (ii) the benefits of high-quality pre-test genetic counselling in supporting families through adverse genomic findings and (iii) the role rapid genomics can play in resolving uncertainty for families and clinicians in circumstances where suspicion of genetic disease exists. This article is accompanied by a Patient Voice perspective written by the child's parents, placing emphasis on the essential role genetic counselling played in their journey.

Genomic sequencing is becoming established as a powerful first-tier test in the diagnosis of rare disease. It is increasingly deliverable with rapid turnaround times, with results available within hours and days for critically ill patients, expediting what would have traditionally been a prolonged or invasive diagnostic odyssey lasting many months or years.[1,2] Beyond diagnostics, genomic sequencing is also increasingly used for screening, in particular for expanded carrier screening (ECS) of couples planning or in the early stages of pregnancy. Prior to the routine clinical use of genomic sequencing, reproductive carrier screening was often limited to, for example, common variants in the *CFTR* gene or gene variants already established within a family. ECS uses genomic sequencing to identify the risk of a couple conceiving a baby affected by a defined panel of recessive and X-linked disease genes. In the screening context, only gene variants where there is strong evidence for pathogenicity are reported. As such, ECS cannot resolve all risk of conceiving a child with a genetic disease.

Here, we present a case of neonatal onset, Type 1 Bartter syndrome (due to compound heterozygous variants in *SLC12A1*) born to parents following low-risk pre-conception ECS. Bartter syndrome is a rare autosomal recessive tubulopathy with five genetic subtypes.[3] Severely affected neonates present with profound polyhydramnios and life-threatening post-natal urinary free water and electrolyte losses requiring aggressive supplementation. The genetic diagnosis was made on day 7 of life using an ultra-rapid, diagnostic genomic testing. On reflection, *SLC12A1* was not included in the gene panel used by the laboratory providing the ECS service. Throughout their journey, the couple was supported by genetic counselling which framed the limitations of the tests provided and supported them through their adjustment to the unexpected diagnosis.

This case highlights the clinical utility of these emerging genomic programs and highlights the importance of genomic literacy for clinicians involved in the assessment of antenatally detected fetal abnormalities. A glossary of novel genomic terms is provided in Table 1.

Case Report

Ahead of trying for their first child, a couple sought referral to a public clinical genetic service for pre-conception carrier screening. There was a personal history of Hashimoto's thyroiditis and a family history of autoimmune lymphoproliferative syndrome for the prospective mother. Her partner was healthy with no family history of genetic conditions. They saw a genetic counsellor who discussed the potential benefits and limitations of the available tests. This included a three gene screen limited to cystic fibrosis, spinal muscular atrophy and fragile X syndrome, and a larger screen of 288 genes associated with predominantly recessive diseases. The couple self-funded the larger screen and received a low-risk result which was returned by the genetic counsellor.

The couple conceived 2 months later. A non-invasive prenatal test returned a low risk for aneuploidies at 10 weeks gestation. The pregnancy remained uncomplicated until 23 weeks gestation when the

Correspondence: Dr Thomas A Forbes, Department of Nephrology, Royal Children's Hospital, 50 Flemington Road, Parkville, Vic. 3052, Australia. Fax: +61 (3) 9345 5611; email: tom.forbes@rch.org.au

Conflict of interest: None declared.

Accepted for publication 2 March 2022.

Table 1 Genomics glossary

Term	Definition
Pre-conception genetic screening (aka reproductive carrier screening)	Prior to conception, a couple undertake genetic testing to determine their risk of conceiving a baby with autosomal recessive or X-linked recessive genetic disease.
Expanded carrier screening	Pre-conception genetic screening has previously been able to inform a couple of their risk of conceiving a baby with (i) a genetic condition that is known to be inherited within their family or (ii) screening for common conditions such as cystic fibrosis, spinal muscular atrophy and Fragile X syndrome. *Expanded* carrier screening is a novel approach that utilises genomic sequencing to screen for hundreds of autosomal recessive and X-linked inherited diseases which are known to cause disease in childhood.
Whole exome sequencing (WES)	Genetic testing by massive parallel sequencing of all the coding regions (exons) of a person's DNA, which typically accounts for 1% of the genome.
Whole genome sequencing (WGS)	Genetic testing by massive parallel sequencing of all the coding regions (exons) and non-coding regions (introns, promoters and others) of a patient's DNA, that is the entire genome.
Trio WES/WGS	Sequencing (either WES or WGS) is simultaneously performed for the child and both parents. This improves efficiency of analysis and reporting by reducing the number of variants to be considered and establishing their inheritance.
Acute care genomics (aka Rapid diagnostic genomics)	Expedited WES or WGS testing performed with turnaround times ranging from 2 to 3 weeks (rapid testing) to <5 days (ultra-rapid testing). Typically, the cost is much higher compared with conventional WES/WGS, which has turnaround times of 3–6 months.

mother developed progressive and painful polyhydramnios (amniotic fluid index up to 63 cm; normal <18 cm) in the context of an anatomically unremarkable fetal ultrasound. Two amnioreductions were performed at 28 weeks draining 4.0 L of amniotic fluid over 3 days. Fluorescent *in situ* hybridisation (for trisomy) and chromosomal microarray were normal. No clear cause for the polyhydramnios was identified. Shortly after the second amnioreduction, onset of premature labour resulted in the delivery of a boy, weighing 1000 g, who developed mild respiratory distress and was transferred to neonatal intensive care.

The baby passed over 6 mL/kg of urine over the first 4 h of life. His serum sodium dropped from 143 to 135 mmol/L and his haematocrit of 0.72 (normal: 0.45–0.65) suggested haemoconcentration from excessive diuresis. He suffered an acute kidney injury (Cr 133 μmol/L, normal 53–97) related to dehydration and gentamicin toxicity. On day 4, he was transferred to a quaternary paediatric hospital and was managed with aggressive intravenous fluids (up to 750 mL/kg/day) and electrolyte supplementation. Bartter syndrome appeared a reasonable differential diagnosis and review of the pre-conception gene panel revealed that only one of the six established genes for Bartter syndrome was included. The couple were again counselled by a clinical geneticist and a genetic counsellor and consented for rapid trio whole genome sequencing (WGS) through the Acute Care Genomics study.[4] Trio WGS identified compound heterozygous, pathogenic variants in *SLC12A1* (c.1432G>A; pGly478Arg and c.1966C>T; pGln656*) with a turnaround of 3 days. These results were returned to the parents by a genetic counsellor and paediatric nephrologist.

As he recovered from his acute kidney injury, the classical Bartter-type biochemical phenotype evolved, including hyponatraemia, hypochloraemia, hypokalaemia and metabolic alkalosis. He received inpatient treatment with gradual transition from parenteral to enteral fluids and nutrition and commenced ibuprofen for prostaglandin synthesis inhibition, a recognised pathogenic phenomenon in Bartter syndrome.[3]

Discussion

Genomic testing is quickly becoming part of mainstream health care. Beyond diagnostic uses, genomic technologies are increasingly being applied in pre-conception screening, providing prospective parents with information on the likelihood of having a child with a genetic condition. Many laboratories offer privately funded pre-conception ECS panels, with no unifying national or international guidance. These tests are increasingly performed on an exome or genome backbone with all variants analysed, but only pathogenic and likely pathogenic variants reported. In this case, however, the disease-causing gene was not included in the screening panel offered. The panel of genes analysed differs between services depending on many internally governed factors including: (i) frequency of disease; (ii) severity of disease; (iii) ability to adequately sequence the gene and (iv) cost among others. Generally, genes chosen for inclusion in ECS are those in which pathogenic variants cause autosomal and X-linked recessive conditions and which result in childhood onset conditions. Only pathogenic and likely pathogenic variants should be reported.[5]

Additionally, genetic disease may arise due to *de novo* (i.e. not inherited) variants and therefore cannot be screened for. Finally, variants of unknown significance may not reach a threshold for reporting in the screening setting, but some will be disease associated. It follows that ECS cannot eliminate all risk of genetic disease in the offspring of parents with low-risk results. It is important that clinicians are aware of these limitations and are comfortable integrating ECS results into their diagnostic formulations, especially in the context of a fetus with classical presentations of genetic disease.

Mackenzie's Mission is an Australian research study examining the utility of ECS for 10 000 couples across Australia with the aim of defining the ideal delivery of ECS to Australian couples. This study was not open at the time our parents were consulted.

The process for selecting the 1300 genes selected for the Mackenzie's Mission panel included review of OMIM and 23 available gene lists by 16 clinical geneticists and a multidisciplinary committee including geneticists, genetic counsellors, an ethicist and the parents of an affected child.[6] Selected genes represent disabling or life-limiting diseases for which early diagnosis and treatment would change outcome or pregnancy planning.[6]

Genomic testing has also established itself as a powerful tool in paediatric rare disease diagnosis, not only ending many a diagnostic odyssey, but also deliverable in 'real time' to influence medical management in critically ill babies.[4,7] The first published feasibility report from the Australian Acute Care Genomics study identified a molecular diagnosis in 51% of critically unwell patients with a median turnaround time of 3 days from sample receipt.[4] This diagnosis facilitated targeted treatment (11%), palliative care decision-making (13%) and focused investigation for known associated features (18%).[4] In our case, Bartter syndrome was astutely recognised as a differential diagnosis from the moment the baby was delivered, despite the low-risk ECS and normal antenatal microarray. Rapid trio WGS was able to quickly secure a post-natal diagnosis, identifying compound heterozygous pathogenic variants in *SLC12A1* (OMIM: 600839).

Whilst in this case the premature delivery occurred too soon to allow a genetic diagnosis to impact immediate management post-delivery, rapid genomic testing on prenatal samples will increasingly inform antenatal diagnoses in future. Hypothetically, an earlier antenatal diagnosis may have allowed earlier specialist consultation and earlier initiation of the extraordinary intravenous fluid administration this baby required (as high as 750 mL/kg/day, normal 60–120) which may have reduced his risk of acute kidney injury. Prospectively, the PreGen Study (www.pregen.neura.edu.au) is an Australian research study that aims to examine the role of genomic sequencing as an antenatal diagnostic tool in the setting of a fetus with a suspected genetic abnormality.

The understanding and perception of the utility of genomic sequencing approaches among paediatricians, midwives and obstetricians is variable.[8–10] Given the rapidly evolving nature of the field, it is imperative that both families and clinicians have a good understanding of the purpose, strengths and limitations of different types of genomic tests so that these are appropriately integrated into clinical care. Additionally, genomic testing should include ready access to clinical geneticists and genetic counsellors, not only in returning prenatal results, but also to support clinicians and families to optimise the investigation of suspected genetic disease in the antenatal and post-natal periods, including the reanalysis of existing genomic data as the clinical situation evolves.

Beyond providing resolution of a genetic diagnosis for our family, rapid genomic testing allowed the clinical team to proceed with confidence in treating the child's Bartter syndrome. This included expediting the collection of international sub-specialist opinions, ruling out differential diagnoses (e.g. other tubulopathies, polyuric recovery phase of acute tubular necrosis), and increased confidence in using medications that were not without risk in a premature neonate (e.g. risk of necrotising enterocolitis and potentiated acute kidney injury with ibuprofen).

The trio rapid WGS in this case was funded through the Acute Care Genomics study, which has been running since 2018 and recruits from all the states and territories.[4] The alternative routine genomic sequencing typically carries a turnaround time of between 3 and 4 months, where the cost has typically been covered by hospitals and clinical services. In Australia, public healthcare system funding is gradually becoming available for routine genomic sequencing from a mixture of federal and state government sources as the evidence for diagnostic yield, clinical utility and cost-effectiveness builds. Currently, the Australian Medicare Benefits Schedule supports genomic testing in children with syndromic and non-syndromic intellectual disability. Additional item numbers for cardiac and renal disorders are expected to become available in the near future providing greater equity of access.

Daniel and Kimberley's reflections on their experience highlight the essential role genetic counselling played throughout their journey, especially with respect to adjusting to the challenging post-natal environment they found themselves in (ref Patient Voice article). This is consistent with recent, published experience of parents receiving genetic counselling for rapid genomic testing.[11] Genetic counsellors possess a skillset that is adaptable to support parents in the critical care setting, and are increasingly playing an extended role as part of rapid genomic testing programs.[12] These supports are crucial as families navigate the complexities of genomic testing, as evidenced by Daniel and Kimberley's experience during the return of genetic results as one of relief and resolution, rather than shock, regret or grief.

Conclusion

The clinical applications of genomic sequencing are rapidly expanding within paediatric and obstetric care. While low-risk ECS reduces the chance of genetic conditions, it does not eliminate it. As for any medical test, clinicians require an understanding of the limitations of genomic sequencing and reporting to prepare families for a variety of potential outcomes. Rapid genomic testing is playing an increasing role in expediting diagnosis and reducing the financial and emotional cost of the diagnostic odyssey in critically unwell infants. Of utmost importance, genetic counsellors are a critical component of any team providing genomic testing, in both the pre- and post-test consultation. Together with the accompanying Patient Voice, this case illustrates the benefits and challenges of clinical genomic testing in resolving an unexpected diagnosis for a critically ill newborn.

Acknowledgements

Trio whole genome sequencing of the patient and parents was performed as part of the Australian Genomics Acute Care study with Human Research Ethics Committee approval HREC/16/MH251, and funding from the Medical Research Futures Fund (GHFM76747). The research conducted at the Murdoch Children's Research Institute was supported by the Victorian Government's Operational Infrastructure Support Program. Open access publishing facilitated by The University of Melbourne, as part of the Wiley - The University of Melbourne agreement via the Council of Australian University Librarians. [Correction added on May 19, 2022, after first online publication: CAUL funding statement has been added.]

References

1 Stark Z, Ellard S. Rapid genomic testing for critically ill children: Time to become standard of care? *Eur. J. Hum. Genet*. 2022; **30**: 142–9.
2 Gorzynski JE, Goenka SD, Shafin K *et al*. Ultrarapid nanopore genome sequencing in a critical care setting. *N. Engl. J. Med.* 2022; **386**: 700–2.
3 Konrad M, Nijenhuis T, Ariceta G *et al*. Diagnosis and management of Bartter syndrome: Executive summary of the consensus and recommendations from the European Rare Kidney Disease Reference Network Working Group for Tubular Disorders. *Kidney Int*. 2021; **99**: 324–35.
4 Australian Genomics Health Alliance Acute Care Flagship. Feasibility of ultra-rapid exome sequencing in critically ill infants and children with suspected monogenic conditions in the Australian public health care system. *JAMA* 2020; **323**: 2503–11.
5 Delatycki MB, Laing NG, Moore SJ *et al*. Preconception and antenatal carrier screening for genetic conditions: The critical role of general practitioners. *Aust. J. Gen. Pract*. 2019; **48**: 106–10.
6 Kirk EP, Ong R, Boggs K *et al*. Gene selection for the Australian Reproductive Genetic Carrier Screening Project ("Mackenzie's Mission"). *Eur. J. Hum. Genet*. 2021; **29**: 79–87.
7 Owen MJ, Niemi A-K, Dimmock DP *et al*. Rapid sequencing-based diagnosis of thiamine metabolism dysfunction syndrome. *N. Engl. J. Med.* 2021; **384**: 2159–61.
8 Stark Z, Nisselle A, McClaren B *et al*. Attitudes of Australian health professionals towards rapid genomic testing in neonatal and paediatric intensive care. *Eur. J. Hum. Genet*. 2019; **27**: 1493–501.
9 Saleh M, Kerr R, Dunlop K. Scoping the scene: What do nurses, midwives, and allied health professionals need and want to know about genomics? *Front. Genet*. 2019; **10**: 1066.
10 Hui L, Szepe E, Halliday J, Lewis C. Maternity health care professionals' views and experiences of fetal genomic uncertainty: A review. *Prenat. Diagn.* 2020; **40**: 652–60.
11 Ayres S, Gallacher L, Stark Z, Brett GR. Genetic counseling in pediatric acute care: Reflections on ultra-rapid genomic diagnoses in neonates. *J. Genet. Couns.* 2019; **28**: 273–82.
12 Brett GR, Martyn M, Lynch F *et al*. Parental experiences of ultrarapid genomic testing for their critically unwell infants and children. *Genet. Med.* 2020; **22**: 1976–85.

Kookaburra by Lux Valentine James (age 8) from Operation Art 2021