Rapid Exome Sequencing for Critically Ill Children: Implementation and Challenges in the Asian Context

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Abstract

Objective: Use rapid next-generation sequencing (NGS) to improve our diagnostic yield in critically ill paediatric patients with suspected genetic disorders in the Asian setting.

Design: A diagnostic study conducted between April 2018 and January 2019.

Methods: Next-generation sequencing was performed with the TruSight One gene panel (targeting 4813 genes) followed by MiSeq sequencing on 10 patients who presented with suspected genetic disorders as assessed by their attending physicians.

Results: In 4 of the 10 cases (40%), a genetic diagnosis was achieved, with one further case diagnosed on re-analysis of data 2 years later. The median turn-around time (TAT) for results was 9.5 working days (range 5-19 days). Challenges faced during implementation included sample availability, managing parental and primary physician expectations, cost of testing, and bioinformatic resources.

Conclusion: RapidSeq is an effective method for diagnosing patients with rare diseases, which aids in shortening the diagnostic odyssey, while allowing clinicians to appropriately tailor management for the underlying disorder, and provide accurate genetic counselling for families. However, challenges such as cost and insurance implications still remain a barrier to more widespread use of genomic testing in the local setting, and continued efforts will be required to optimise RapidSeq for use in paediatric patients in the ICU.

Introduction

Genetic conditions are a major cause of morbidity and mortality in children, and are estimated to affect 2–3% of all newborns[1; 2]. There is a high burden of genetic disorders in patients admitted to the intensive care unit (ICU), ranging from 45–56%[3; 4], and delayed definitive diagnoses with a long diagnostic odyssey often contribute to increased healthcare costs[5]. The integration of genomic technologies is transforming healthcare by allowing provision of optimal clinical care for affected children with timely diagnoses, facilitating a shift from empiric treatment to definitive management of an identified disorder when feasible[4–8]. Increasingly, the application of clinical exome sequencing in the paediatric intensive care setting has been gaining traction, where a short turnaround time (usually within two weeks) coupled with a relatively high diagnostic yield (30%-40%) allows for significant changes in the medical management of patients with rare diseases[4–6; 9; 10], and, at the same time, provides patients and their families an opportunity to get answers.

The use of rapid whole genome sequencing (rWGS) was first shown by Saunders et al[6] in 2012, which identified known molecular diagnoses in four of five affected individuals in the neonatal intensive care unit in ~ 50 hours. Farnaes et al[5] (2018) subsequently showed the improved clinical utility of rWGS in acutely ill infants compared to standard genetic tests, with significant cost savings. These findings were replicated in the Australian Public Health Care System[3], which found an established molecular

diagnosis in 51% of their cohort, in which a further 76% had an impact on their clinical management. Clinical utility of optimized trio genome sequencing in critically ill infants in China reported a diagnostic yield of 47.7%[11], but otherwise, data from other Asian populations are lacking.

The Singapore Undiagnosed Disease Programme was set up in 2014 to utilize next generation sequencing (NGS) to aid diagnosis and management of patients with rare disorders[12; 13]. As a follow up to the research program, in April 2018, we worked on translating the research into a rapid exome sequencing (RapidSeq), focusing on known Mendelian genes, for critically-ill patients admitted to the neonatal or children's intensive care units (NICU/CICU). The aim was to provide a genetic diagnosis within 14 days for critically-ill patients in the NICU/CICU who are suspected to have a genetic disorder. We hypothesised that a prompt diagnosis would guide management of the patient. In this study, we present our framework as well as initial results in implementing RapidSeq and highlight the lessons learnt and challenges faced, some of which are unique in the Asian context. More importantly, this framework for delivering RapidSeq was formulated as a continual learning cycle where implementation is monitored and modified over time with emerging evidence.

Study Design, Participants And Settings Overview

This study is a prospective, observational study of the utility of genomics in critically ill children admitted to KK Women and Children's Hospital's (KKH) intensive care units. Ethics approval was obtained by the SingHealth Central Institutional Review Board. Using existing hospital services for clinical testing, our team comprised of a clinical research coordinator, laboratory scientists for sample processing, bioinformaticians, genetic counsellors (GC), a genetics specialty nurse, and a team of clinical geneticists to oversee the logistics and implementation. The overall process from enrolment to results analysis and disclosure is displayed in Fig. 1.

Inclusion/ Exclusion criteria and clinical evaluation

Patients were eligible if admitted to the NICU/CICU and/or had been referred to the clinical genetics service for a suspected genetic condition, or needed an urgent result e.g., for prenatal care, and deemed suitable for testing by the clinical genetics team. Patients were ineligible if (1) a secure clinical diagnosis (such as Down syndrome) was made or previously known; (2) an underlying genetic etiology was considered unlikely or (3) parents unavailable or declined to participate.

Clinical features were characterized by the attending clinical geneticist, with tentative clinical diagnoses established where appropriate. For patients with a non-specific diagnosis that might be caused by genomic imbalance, chromosomal abnormalities were ruled out using karyotyping and/or chromosomal microarray analysis, if indicated. Individuals who fulfilled inclusion and exclusion criteria were then recruited, and pre-test counselling provided by a GC. Informed consent was obtained from patient's parents.

Sample collection and DNA extraction

Once informed consent was obtained, the GC or specialty nurse would liaise with the intensive care team for timely collection of appropriate samples for DNA extraction. Where possible, trio samples (proband and biological parents) were obtained. While blood was the preferred sample type, if blood was not suitable (e.g. recent blood transfusion), alternative sources such as skin biopsy for fibroblast culture and DNA extraction were done. Saliva samples or buccal swabs were deemed unsuitable for this study to yield a sufficient amount of DNA for testing[14], as recruited children were often critically ill and intubated or unable to cooperate with spitting. Samples were then sent to the laboratory for RapidSEq. DNA was isolated using Gentra Puregene Blood Kit (Qiagen, USA) as per manufacturer's protocol.

Sequencing, bioinformatic analysis and variant curation

Targeted sequencing of exonic regions of 4,813 genes associated with human diseases (also referred to as the clinical exome) was performed using the Illumina TruSight One sequencing panel and on the MiSeq Sequencer (Illumina, San Diego, USA). Variant calls were made using the MiSeq Reporter pipeline (Illumina, San Diego, USA) and aligned to human reference GRCh37/hg19. Variant annotation, filtration and prioritisation were performed using wANNOVAR[15] and Illumina Variant Studio (Illumina, San Diego, USA). Variants were checked against those in dbSNP[16], 1000 Genomes[17], Exome Aggregation Consortium (ExAC)[18] or Genome Aggregation Database (gnomAD)[19], ClinVar[20] and Human Gene Mutation Database (HGMD)[21] for information on frequencies and previously reported findings in patients. Missense variants were further evaluated using in silico tools (SIFT[22], Polyphen2[23] and Mutation Taster[24]) to predict the effect of the specific substitution on protein function.

Potential variants were reviewed in conjunction with the clinical genetics team. Where possible, we selected genes of interest (Supplementary Table 1) that correlated with the patient's phenotype for first pass analysis, and also used ClinVar (pathogenic/ likely pathogenic variants) as an additional filter. If a genetic variant was identified to be consistent with the patient's primary phenotype, it was confirmed by Sanger sequencing in the index patients as well as parents, especially for those families where only proband DNA was sequenced, to determine the origin as well as phase of the variant(s). Variants were classified according to the published guidelines of the American College of Medical Genetics and Genomics (ACMG) and the Association of Molecular Pathology (AMP)[25]. A clinical report was then generated, which was shared with the primary care team and the patient's family with a target turnaround time of 14 days.

Results

Participant demographics

From April 2018 to January 2019, 10 cases were enrolled into RapidSeq, ranging from 3 days to 4.5 months old. Seven were patients from NICU, 2 were patients from CICU, and 1 was a stored fetal DNA sample where the mother was 12 weeks pregnant with a second pregnancy. Nine of the 10 patients were of Chinese ethnicity, while 1 was of Filipino and Japanese descent (Table 1).

In 4 of the 10 cases (40%) a genetic diagnosis was achieved (Table 4), with one further case diagnosed on re-analysis of data 2 years later. For 3 of the 4 cases, the diagnosis led to changes being implemented in the patient's management plan. The median turn-around time (TAT) for results was 9.5 working days (range 5-19 days) (Table 2).

Diagnoses and clinical actionability

For cases 2, 6 and 10, the discovery of an underlying unifying diagnosis provided more clarity to the patient's care team, allowing more targeted management and surveillance for underlying complications. For example, case 6 had a *de novo* known pathogenic mutation C>G in the *SAMD9* gene, consistent with a diagnosis of MIRAGE (Myelodysplasia, Infection, Restriction of growth, Adrenal hypoplasia, Genital phenotypes and enteropathy) syndrome, which is consistent with the patient's clinical presentation. Prior to diagnosis, the child was being managed by multiple specialists, including the neonatologist, endocrinologist, and haematologist, with multiple investigations done to assess for the etiology of her underlying manifestations. With identification of her diagnosis, this allowed for a more holistic and targeted management of her underlying condition, and furthermore also facilitated surveillance for other complications, such as for haematological malignancy and immunodeficiency.

Challenges faced

RapidSeq is resource-intensive, requiring the support of a multidisciplinary team[3] including the intensive care physician, medical subspecialists, clinical geneticists, GCs, laboratory scientists, and bioinformaticians simultaneously. Facilitating RapidSeq requires effective coordination between the clinical and laboratory team, and workflows were refined throughout the study period to optimize timely processing of the results. Factors contributing to prolonged turnaround time included difficulties with pre-test counselling, sample limitations, and bioinformatics processing.

Pretest genetic counselling

In critically ill patients, parents are often overwhelmed by the ongoing medical issues and status of the child, experiencing feelings of anxiety, anger, depression and increased stress. This can sometimes impede pre-test counselling, affecting the ability of parents to process the multitude of information presented to them and hence give informed consent. Furthermore, given the absence of genetic non-discrimination regulations in Singapore[26], concerns about a genetic diagnosis affecting insurance is commonly a barrier to testing locally, often delaying initiation of testing until insurance concerns are sorted out. These can hamper the use of genomic sequencing as a diagnostic tool for these patients, given the concerns for secondary findings, which has been noted at 1.6% in our local cohort[27]. For the purposes of this pilot, we chose to only report primary findings and not incidental or secondary findings. Interestingly, in our cohort, none had a delay in initiating testing due to insurance concerns, possibly due to the urgency of diagnosis taking precedence over concerns about insurance implications in a critically ill patient.

Availability of appropriate sample for DNA extraction

Sample collection can also be delayed due to acute medical events in the patient, such as the need for blood transfusions taking priority to sample collection, hence alternative means should be sought. For case 6, the patient had received a packed cell and platelet transfusion 3 and 9 days prior to recruitment. In our institution, it is recommended to wait for 2 weeks following the last transfusion before collection of blood sample to prevent leukocyte contamination. As such, the patient underwent a skin biopsy for skin fibroblast culture and subsequent DNA extraction, adding an additional 11 days before DNA was ready for analysis.

Managing parental and primary physician expectations

Although obtaining a genetic diagnosis often has implications in the management of a critically ill patient, parents, and even primary physicians, can sometimes have raised expectations of the test and its impact on the child's management and prognosis, placing unrealistic expectations on the outcome of the test. It is important to manage parental (and primary physician) expectations of possible result outcomes, which can range from clear cut diagnoses, to variants of uncertain significance, incidental findings, or even a negative result. Case 9 was a preterm infant born at 34+4 weeks with multiple congenital anomalies including tracheo-esophageal fistula, anorectal malformation, left congenital talipes equinovarus, right multicystic dysplastic kidney and low-lying cord. He underwent RapidSeq at day 3 of life which returned negative; this initially made it difficult for parents to understand the extent of his medical condition, as they had hoped that with continued intervention, he would go on to be a normal child. It was then important to communicate to them that in spite of a negative genetic test, the child still had multiple congenital abnormalities that would require long term follow up and medical management.

Turnaround time

The median TAT from sample receipt to provisional results in our study was 9.5 days, with a range of 5-19 days. All patients except case 6 had a TAT <14 working days, with the reason for delay being due to the increased time required for DNA extraction from skin fibroblasts.

Cost of testing

At present, we calculate the cost of RapidSeq to be SGD$6000 (USD$4500). Processes will need to continue to be improved to lower costs to reduce the barriers to testing and keep testing sustainable and feasible.

Bioinformatic resources

In view of cybersecurity concerns, computers in the Singapore public healthcare sector do not have internet access. Cloud transfer of genomics data to the bioinformatician team at a different physical location is thus not possible, and the genomics data has to be transferred via a physical hard disk that requires effective coordination between the laboratory and the bioinformatics team. The large amount of

data generated from the RapidSeq also requires specific informatics needs and tools that are in accordance with clinical testing standard for data management, storage, analysis and archiving. We are exploring alternative options of a cloud-based system that is compatible to the intranet across different public healthcare institutions in Singapore to allow for the continuous provision of data transfer that is necessary in RapidSeq.

Limitations of testing

Bioinformatics filtering process

There are also limits to RapidSeq, whereby only the clinical exome, targeting known genes at that point in time was targeted, or limits with the bioinformatics filtering process. Case 7 was a term infant with multiple congenital anomalies including complex ventriculomegaly with Dandy-Walker malformation, left-sided congenital diaphragmatic hernia, retinal coloboma, and dysmorphic features and was recruited at day 10 of life. However, results returned negative, with parents continuing to hope for active treatment for as long as genetic testing was inconclusive or did not show a lethal genetic condition. She went on to have whole-exome sequencing which found 2 variants of uncertain significant in *WDR81*, which was associated with congenital hydrocephalus type 3. These 2 variants were initially picked up on RapidSeq, but subsequently filtered out, possibly as the variants were of uncertain significance and, furthermore, only some of the child's features could be explained by it. The child stayed in hospital from birth till initial discharge at 10 months old, and underwent multiple operations and procedures, of which her clinical course was complicated by multiple infections. After discharge, she continued to have multiple readmissions before her subsequent demise at 15 months old.

Lack of local reference genomic databases

Our ability to accurately classify variants in our cohort is also challenged by the fact that Asians are under-represented in population and clinical variant databases (e.g. gnomAD and Clinvar), which has resulted in patients of Asian ancestry being more likely to receive ambiguous genetic test results or variants of uncertain significance[28]. A recent local study of patients with suspected undiagnosed genetic conditions has shown that up to 61% of the variants seen in this multiethnic Asian population are novel[12]. We used trio-based sequencing as a way to improve our variant filtering process as illustrated by reduced number of candidate variants in Table 3.

Evolution of clinical phenotype and need for reanalysis

Furthermore, correlation with clinical phenotype may also be difficult, especially in the paediatric setting, whereby clinical features may develop with age and be less recognizable when young, which has been reported in previous studies[29]. This is seen in our cohort with case 8, a full-term neonate who presented with recurrent apneas, and for whom RapidSeq returned negative; the child was started on anti-epileptic medications, with a presumptive diagnosis of neonatal seizures and remained well. Two years later, the child was noted to have disproportionate short stature, and re-analysis of RapidSeq data showed a

pathogenic variant in the *FGFR3* gene, consistent with a diagnosis of hypochondroplasia. This variant had been found in the initial analysis but decision had been made not to report it as clinically the child did not have any features of hypochondroplasia, and incidental findings were chosen not to be reported for this research. This emphasizes the importance of re-analysing data from unsolved cases at a later timepoint, which could uncover the presence of variants in newly discovered genes or new phenotype-genotype associations[30-33].

Discussion

We have presented results from 10 probands who underwent RapidSeq in our institution. Our diagnostic rate was 40%, with changes in management being seen in 3 out of 4 of the cases. Our results emphasize the clinical utility of RapidSeq in critically-ill patients. A positive genetic diagnosis can play a valuable role in guiding clinical care management and therapeutics. This can result in benefits such as treatment modification, initiating a new treatment, or surfacing the need to involve other specialists in the clinical care of the child. It can also guide the care team in potentially reducing unnecessary interventions, such as stopping futile treatment, or precluding a potentially invasive measure. In some cases, a diagnosis with a dire prognosis can help to bring closure, and enable the care team and family to discuss treatment limitations and palliative care options. RapidSeq shortens and eases the diagnostic odyssey in the patient, decreasing the emotional and financial burden on patients and the healthcare system. There is also impact on the family in terms of genetic counselling, about the risk of recurrence in future pregnancies, for the same genetic disorder.

While genomic sequencing in patients with rare diseases is increasing, there are still multiple challenges to overcome. These include the concern for higher cost[34–36], insurance discrimination[26], need for bioinformatics resources to process and store large amounts of data, and the managing of parental expectations in an emotionally trying period. It is also important to recognize the limitations to genetic testing that is still a rapidly evolving field. As seen, 60% of our cohort received no diagnosis initially, however, with re-analysis of sequence data, a further patient received a diagnosis improving our yield to 50%. Where a patient's condition remains undiagnosed, re-analysis of data and other forms of tests, including whole genome sequencing, can be offered as per their clinical indication, to better understand their conditions. The successful implementation of a RapidSeq program will require continued efforts to improve clinician awareness and recognition, and improved diagnostic tools to aid in the evaluation of variants.

Conclusion

RapidSeq is an effective method for diagnosing patients with rare diseases, which aids in shortening the diagnostic odyssey, while allowing clinicians to appropriately tailor management for the underlying disorder, and provide accurate genetic counselling for families. However, challenges such as cost and insurance implications still remain a barrier to more widespread use of genomic testing in the local

setting, and continued efforts will be required to optimise RapidSeq for use in paediatric patients in the ICU.

Abbreviations

CICU	Children's Intensive Care Unit
GC	Genetic Counsellor
ICU	Intensive Care Unit
KKH	KK Women and Children's Hospital
NGS	Next-generation sequencing
NICU	Neonatal Intensive Care Unit
rWGS	Rapid whole genome sequencing
RapidSeq	Rapid Exome Sequencing
TAT	Turn-around time

Declarations

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