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SHORT REPORT

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Rapid exome sequencing as the first-tier investigation for diagnosis of acutely and severely ill children and adults in Thailand

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Abstract

The use of rapid DNA sequencing technology in severely ill children in developed countries can accurately identify diagnoses and positively impact patient outcomes. This study sought to evaluate the outcome of Thai children and adults with unknown etiologies of critical illnesses with the deployment of rapid whole exome sequencing (rWES) in Thailand. We recruited 54 unrelated patients from 11 hospitals throughout Thailand. The median age was 3 months (range, 2 days–55 years) including 47 children and 7 adults with 52% males. The median time from obtaining blood samples to issuing the rWES report was 12 days (range, 5–27 days). A molecular diagnosis was established in 25 patients (46%), resulting in a change in clinical management for 24 patients (44%) resulting in improved clinical outcomes in 16 patients (30%). Four out of seven adult patients (57%) received the molecular diagnosis which led to a change in management. The 25 diagnoses comprised 23 different diseases. Of the 34 identified variants, 15 had never been previously reported. This study suggests that use of rWES as a

first-tier investigation tool can provide tremendous benefits in critically ill patients with unknown etiology across age groups in Thailand.

KEYWORDS
acutely ill patients, adults, rapid exome sequencing, Thai, Thailand

1 | INTRODUCTION

In critical care settings, rapid diagnostic test results are crucial for making definitive diagnoses which can lead to targeted treatment and save lives. Genetic diseases account for 14% and 1.9% of all diseases in newborns[1,2] and adults[3] admitted to intensive care units (ICUs), respectively. With the advancement of molecular technologies, rapid next generation sequencing (rNGS) has been described as a useful diagnostic tool for critical care settings since 2012[4] as it possesses a high diagnostic yield which can impact clinical management.[4-11] Due to the rapid improvement in cost-effectiveness, turnaround time and accuracy, rNGS, either rapid whole genome sequencing (rWGS) or rapid whole exome sequencing (rWES), has been introduced as a first-tier diagnostic tool for critically ill newborns and children.[12]

Whereas these technologies have been performed and evaluated mostly in children living in the United States, Europe, and Australia,[4-7,9] and very recently in Taiwan,[8] Hongkong,[10] and China,[11] their beneficial impact on clinical management in people from different ethnicities and geographic areas need to be examined.[13] The mutational spectra and positions of the same disease in different ethnicities are often dissimilar. Moreover, different countries have different patterns of illnesses. Unlike developed countries, accidents, ingestion of toxins, and malnutrition remain common causes of ICUs admission in Thailand. This may lower the yield of a diagnostic tool for genetic diseases.

To our knowledge, data from rWES in critically ill adults are yet unavailable. Diseases affecting children and adults are distinctive. Each genetic disease has its own phenotypic spectrum and age of onset. Previous studies of rapid genomic testing have primarily targeted only infants and children in ICUs.

Differences in genetic background, local environmental characteristics, and age of onset may affect the diagnostic yield and utility of rWES. This study aims to evaluate the clinical impact of rWES in acutely ill Thai children and adults with no explainable causes across multiple centers in Thailand.

TABLE 1 Diagnostic results of rWES by demographic and clinical characteristics

		Diagnostic (n = 25)	Negative (n = 29)	Total (n = 54)
Gender	Female	11 (42%)	15 (58%)	26
	Male	14 (50%)	14 (50%)	28
Age group	<1 month	6 (38%)	10 (62%)	16
	1 month–1 year	13 (62%)	8 (38%)	21
	1–18 years	2 (20%)	8 (80%)	10
	>18 years	4 (57%)	3 (43%)	7
Setting	ICU	19 (45%)	23 (55%)	42
	In patient department	5 (42%)	7 (58%)	12
rWES categories	Singleton	3 (38%)	5 (62%)	8
	Duo	1 (100%)	0 (0%)	1
	Trio	21 (48%)	23 (52%)	44
	Quad	0 (0%)	1 (100%)	1
Primary involved system	Multiple anomalies	1 (50%)	1 (50%)	2
	Pulmonary	1 (50%)	1 (50%)	2
	Cardiology	1 (33%)	2 (67%)	3
	Nephrology	1 (50%)	1 (50%)	1
	Gastrointestinal	3 (38%)	5 (62%)	8
	Neurology	8 (44%)	10 (56%)	18
	Immunology	1 (25%)	3 (75%)	4
	Hematology/oncology	5 (83%)	1 (17%)	6
	Dermatology	2 (100%)	0 (0%)	2
	Endocrinology	1 (17%)	5 (83%)	6
	Psychiatry	1 (100%)	0 (0%)	1

TABLE 2 Acute precision medicine interventions in 25 patients receiving genetic disease diagnoses and the resultant changes in management and/or outcome

Patient No.	Gender, age at testing	Primary system/main clinical presentation	Causal gene (disease/OMIM)	Management change	Outcome change
1	M, 24 years	Psychiatry/schizophrenia and catatonia	*MMACHC* (Methylmalonic aciduria and homocystinuria, cblC type/277 400)	Yes	Yes
2	F, 5 years	Neurology/coma and acute liver failure after episode of influenza A infection	*OTC* (Symptomatic carrier of ornithine transcarbamylase deficiency/311 250)	Yes	No
3	M, 9 months	Neurology/drowsiness, hepatomegaly, hypoglycemia, hyperlipidemia	*HMGCS2* (HMG-CoA synthase-2 deficiency/605 911)	Yes	Yes
4	F, 9 months	Neurology/drowsiness, severe metabolic acidosis	*HMGCS2* (HMG-CoA synthase-2 deficiency/605 911)	Yes	Yes
5	F, 1 month	Neurology/intractable seizure	*SCN1A* (Epileptic encephalopathy, early infantile, 6/607 208)	Yes	Yes
6	M, 2 months	Neurology/intractable seizure	*KCNT1* (Epileptic encephalopathy, early infantile, 14/614 959)	Yes	Yes
7	F, 3 days	Neurology/intractable seizure	*KCNQ2* (Epileptic encephalopathy, early infantile, 7/613 720)	Yes	Yes
8	F, 3 months	Neurology/intractable seizure	*KCNQ2* (Epileptic encephalopathy, early infantile, 7/613 720)	Yes	Yes
9	M, 1 month	Neurology/intractable seizure	*ALDH7A1* (Epilepsy, pyridoxine-dependent/266 100)	Yes	Yes
10	F, 2 days	Cardiology/atrial septal defect, dilated aortic sinus, respiratory distress, multiple anomalies (arachnodactyly, widely spaced eyes), history of maternal aortic root dilatation	*TGFBR1* (Loeys-Dietz syndrome 1/609 192)	Yes	No
11	M, 3 months	Multiple anomalies/truncus arteriosus type 1 with cleft palate	*CHD7* (CHARGE syndrome/214 800)	Yes	No
12	M, 9 months	Pulmonology/recurrent pneumonia monthly, respiratory failure with failure to thrive	*CFTR* (Cystic fibrosis/219 700)	Yes	Yes
13	M, 3 days	Hematology, oncology/hydrops fetalis	*SPTB* (Anemia, neonatal hemolytic, fatal or near-fatal/617 948)	Yes	No
14	M, 45 years	Hematology, oncology/atypical lymphoma, hepatomegaly, aortic root dilatation, multiple anomalies	*DNMT3A* (Tatton-Brown-Rahman syndrome/615 879)	Yes	No
15	F, 19 years	Hematology, oncology/ pancytopenia with pyoderma gangrenosum	*GATA2* (Immunodeficiency 21/614 172) (Leukemia, acute myeloid, susceptibility to/601 626) *PTPN11* (Noonan syndrome 1/163 950)	Yes	No
16	F, 8 months	Hematology, oncology/ thrombocytopenia with hepatomegaly	*PRF1* (Hemophagocytic lymphohistiocytosis, familial, 2/603 553)	No	No
17	M, 7 months	Hematology, oncology/seizure with rhabdoid tumor	*SMARCB1* (Rhabdoid tumor predisposition syndrome 1/609 322)	Yes	Yes
18	M, 2 months	Nephrology/severe persistence hyperkalemia	*NR3C2* (Pseudohypoaldosteronism type I/177 735)	Yes	Yes

TABLE 2 (Continued)

Patient No.	Gender, age at testing	Primary system/main clinical presentation	Causal gene (disease/OMIM)	Management change	Outcome change
19	M, 47 years	Gastroenterology/liver failure, jaundice	*ATP7B* (Wilson disease/277 900)	Yes	Yes
20	F, 8 months	Gastroenterology/liver failure, jaundice	*JAG1* (Alagille syndrome 1/118 450)	Yes	Yes
21	F, 3 months	Gastroenterology/diarrhea, multiple organ dysfunction, hemolytic anemia	*NLRC4* (Autoinflammation with infantile enterocolitis/616 050)	Yes	Yes
22	M, 16 days	Endocrinology/Persistent hyperinsulinemic hypoglycemia	*ABCC8* (Hyperinsulinemic hypoglycemia, familial, 1/256 450)	Yes	Yes
23	M, 7 months	Immunology/Recurrent infection	*RAG1* (Severe combined immunodeficiency, B cell-negative/601 457)	Yes	Yes
24	M, 15 days	Dermatology/multiple skin blister since birth	*COL7A1* (Epidermolysis bullosa dystrophica, AR/226 600)	Yes	No
25	F, 2 days	Dermatology/multiple skin blister since birth	*KRT5* (Epidermolysis bullosa simplex, Dowling-Meara type/131 760)	Yes	No
Total			Diagnosed cases 25/54 (46%)	Changes in management 24/54 (44%)	Changes in outcome 16/54 (30%)

2 | MATERIALS AND METHODS

Participants were recruited from 11 tertiary hospitals in Thailand from January 2018 to August 2020. Eligibility criteria included[1] patients admitted to ICUs or inpatient wards with seriously ill conditions, and[2] no obvious causes such as a road accident. Primary physicians at each site served as recruiters. Research Electronic Data Capture (REDCap) software was used to facilitate the timely exchange of information between the clinical and laboratory teams. The rWES report for each case was distributed to the primary hospital, and the summary statistics were sent to the core team at the King Chulalongkorn Memorial Hospital (KCMH) in Bangkok. The outcomes were the molecular diagnostic yield and the change in clinical management.

The study was approved by the Institutional Review Board at KCMH. Informed consent was obtained from each patient's guardian. rWES was performed in the proband and available parents. Genomic DNA was extracted from peripheral blood leukocytes. The DNA samples were prepared as an Illumina sequencing library enriched by TruSeq® Exome Kit (Illumina) and was sequenced onto NextSeq 500 System (Illumina). The Burrows-Wheeler Aligner was used to map raw data to the human reference genome version 19. GATK and HaplotypeCaller were used for variant calling. SNVs and Indels were annotated using SnpEff, dbpSNP 142, ClinVar, 1000 Genomes, and ESP. Variants relevant to the patient phenotype were classified based on the standards and guidelines of the American College of Medical Genetics and Genomics.[14] Variants were considered novel when they were not reported in the Clinvar, 1000 Genomes, gnomAD and HGMD databases.

3 | RESULTS

Of the 54 unrelated patients, rWES was performed on 44 trios (child and parents) and 1 quad (parents and two affected siblings), 1 mother–infant duo, and 8 singletons (6 adults and 2 children). All patients self-reported Thai ancestry. Consanguinity was denied by all families. A total of 42 out of 54 (78%) were in ICUs. (Table 1) The most common presentation was neurological symptoms (33%). The median age was 3 months (range: 2 days–55 years old). Seven adults (>18 years of age) were recruited (4 males and 3 females). The median turnaround time of rWES was 12 days (range 5–27 days). The fastest turnaround time was 5 days (Patient 4).

Of the 54 patients, rWES could provide a molecular diagnosis in 25 patients producing a diagnostic yield of 46% (Table 2). All 25 diagnoses could explain the phenotype comprising 23 genetic diseases. Of the 34 identified genetic variants, 15 were novel pathogenic or likely pathogenic in known disease genes (Table S1).

The one-month-to-one-year age group had the highest diagnostic yield of 62% (13 of 21). The number of patients in the ICU setting who received a diagnosis from rWES was similar to the in-patient department (IPD, non-ICU) setting (45% and 42%). Trio rWES had a higher diagnostic yield of 48% (21/44) compared to 38% (3/8) of the singleton rWES. Presentation in hematology/oncology received the highest percentage yield (5/6 = 83%).

Specific changes in medical or surgical treatment occurred as a result of molecular diagnoses (clinical utility) in 24 of 54 patients (44%). Six medications were started in four patients (Patients 1, 10,

12, 19). In a total of seven patients (patients 3, 4, 11, 12, 20, 24, 25) (7/25, 28% of diagnosed), surgical procedures were changed. In total, rWES-diagnosis was judged to have prevented morbidity in 16 patients (16/54, 30%).

4 | DISCUSSION

Evidence for clinical utility of rWES in patients with acute and severe illnesses of unknown causes, has been limited in populations of non-European ancestry in developing countries. This study reports the impact of rWES on clinical outcomes and healthcare management in newborns, children and adults of Thai ethnicity. A total of 54 patients ranging from 2 days to 55 years old were recruited. The median rWES diagnosis turnaround time of 12 days (range 5–27 days) in our study is within the general range from previous reports (<1–109 days) (Table S2). Interpretation of the variants is the rate-limiting step in our study. Complexity of the clinical manifestations, genotype–phenotype correlation, availability of the parental blood samples, and experience of the variant scientists and the team (faster turn-around time toward the end of the project) play a role in the turn-around time. The diagnostic yield of 46% is also comparable with previous data reporting a median of 45% (range 19%–72%). Further, many genetic diseases diagnosed in our patients were similar to other ethnicities, such as illnesses caused by *KCNQ2*, *CHD7*, and *OTC* mutations (Table 2). Notably, 15 out of the 34 identified etiologic genetic variants were novel mutations in known disease genes suggesting a different mutational spectrum in the Thai population. Specific changes in treatment occurred as a result of molecular diagnoses in 24 of 54 patients (44%) which is well-matched with published rates from previous studies (median 30%, range 12.5%–61%). Our findings support the application of rWES as a first-tier diagnostic test in acutely ill patients in Thailand.

Although genetic diseases in adult patients comprised only 1.9% of total hospitalized patients,[3] our study demonstrated that even the adult population may also benefit from rWES when they are in critical stages of undiagnosed genetic diseases. For instance, patient 1, a 24-year-old man presented to the psychiatry department with schizophrenia and catatonic stage. The rWES revealed a diagnosis of methylmalonic aciduria and homocystinuria, cblC type.[15] Treatment with hydroxocobalamin and betaine resulted in marked improvement in his neurological and psychiatric symptoms. He also has an older sister who suffered from bipolar disorder. His sister then decided to obtain DNA sequencing which revealed the same variants. After treatment, both are now healthy and able to go back to work. As compared with the pediatric population, the diagnostic yield, turnaround time, and outcome alternation in the adult population did not differ significantly (Table S3). This suggests how use of rWES in acutely ill adults might yield benefits. Further studies with more patients are needed for evaluating the usefulness of rWES in adults.

In summary, while our study had a small size of population, it suggests the likelihood of using rWES as the first-tier investigation in acutely ill Thai patients with unknown causes served by the Thai public health care system. Both a cost-effectiveness study of rWES and funding of long-term patient follow-up study to increase benefit evaluation are warranted.

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CONFLICT OF INTEREST

The authors declare no potential conflict of interest.

PEER REVIEW

The peer review history for this article is available at https://publons.com/publon/10.1111/cge.13963.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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