

Contents lists available at [ScienceDirect](https://www.sciencedirect.com)

The Lancet Regional Health - Western Pacific

journal homepage: www.elsevier.com/locate/lanwpc

Research paper

Rapid whole-exome sequencing facilitates precision medicine in paediatric rare disease patients and reduces healthcare costs

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ARTICLE INFO

Article history:

Received 30 April 2020

Revised 8 June 2020

Accepted 11 June 2020

Keywords:

Rapid whole-exome sequencing

Paediatric

Children

Rare disease

Precision medicine

Healthcare cost-savings

ABSTRACT

Background: Rapid whole-exome sequencing (rWES) offers the potential for early diagnosis-predicated precision medicine. Previous evidence focused predominantly on infants from the intensive care unit (ICU). This study sought to examine the diagnostic and clinical utility, and the economic impact on clinical management of rWES in patients beyond infancy and ICU setting.

Methods: rWES was performed on a prospective cohort of patients with suspected monogenic disorder referred from territory-wide paediatric ICUs and non-ICUs in Hong Kong urging for rapid genetic diagnosis. All eligible families were invited. We aimed to achieve a rapid turnaround time (TAT) of 14 days. Clinical utility and costs associated with clinical management were assessed in diagnosed cases. Actual quantitative changes in healthcare utilisation were compared with a counterfactual diagnostic trajectory and/or with matched historical control whenever possible.

Findings: rWES were offered to 102 families and 32/102 (31%) patients received a molecular diagnosis, with a median TAT of 11 days. Clinical management changed in 28 of 32 diagnosed patients (88%), including but not limited to modifications in treatment, avoidance of surgeries, and informing decisions on redirection of care. Cost analysis was performed in eight patients. rWES was estimated to reduce hospital length of stay by 566 days and decrease healthcare costs by HKD\$8,044,250 (GBP£796,460) for these eight patients. The net cost-savings after inclusion of rWES costs were estimated to be HKD\$5,325,187 (GBP£527,246).

Interpretation: This study replicates the diagnostic capacity and rapid TAT of rWES in predominantly Chinese patients, and demonstrates diagnosis-predicated precision medicine and net healthcare savings. Findings were corroborated by evidence from multinational cohorts, combined as part of a meta-analysis. rWES merits consideration as a first-tier diagnostic tool for patients with urgent needs in the clinical setting.

Funding: Health and Medical Research Fund, HKU Seed Fund for Basic Research, The Society for the Relief of Disabled Children, and Edward and Yolanda Wong Fund.

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<https://doi.org/10.1016/j.lanwpc.2020.100001>

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Research in context

Evidence before this study: Recently, there is an increasing interest in the use of rapid whole-exome sequencing (rWES) and rapid whole-genome sequencing (rWGS) in critically ill children as they offer a much faster turnaround time (TAT) in days, as contrasted to the standard WES and WGS, or a succession of different conventional diagnostic tests. The rapid diagnosis has the potential for diagnosis-predicated precision medicine, which is critical to reduce suffering, morbidity, and mortality. However, evidence regarding the clinical utility and economic impact of rWES and rWGS is inadequate. We searched PubMed with the search terms “whole exome sequencing” or “whole genome sequencing”, and “rapid” or “critical” or “intensive” or “ICU” to identify relevant studies. There were no language and date restrictions. Only findings from rWES / rWGS were examined. Studies targeting on one specific group of disorder were excluded. The diagnostic capacity and TAT of rWES and rWGS were shown to be similar. Most studies included a small number of patients being offered rWES or rWGS ($n < 50$), with a focus on (i) infants younger than one year of age; and/ or (ii) patients recruited from intensive care units, with inadequate information on management implications and the associated costs.

Added value of this study: To our knowledge, this is the largest prospectively ascertained cohort of using rWES in patients with suspected monogenic disorder that focused on the impact of clinical management and its actual cost-savings. Our study demonstrated rWES achieved a diagnostic yield of 31% with a median TAT of 11 days, comparable to international studies. Importantly, rWES aided clinical management in 88% of diagnosed patients in this cohort, which is higher than most of the published studies. We also demonstrated rWES reduced healthcare expenditure and achieved net healthcare cost-savings in the clinical setting. This study extends the body of evidence in predominant Chinese patients beyond infancy and beyond intensive care.

Implications of all the available evidence: The clinical implication of rWES was compared with and corroborated by findings from cohorts from other countries, combined as part of a meta-analysis. Findings from this study and all available evidence shed light on the consideration of integrating rWES into clinical workflows to enable precision medicine in the paediatric and adolescent population. Further research is needed to identify the long-term clinical and economic impact from the individual, family, and healthcare system perspective.

odyssey [7]. A timely diagnosis has the potential to initiate or alter clinical management promptly and profoundly, which may diminish disease severity, slow down disease progression, avoid unnecessary hospitalisation and healthcare utilisation, improve patient's clinical outcome, and potentially be life-saving [8,9]. In addition, early and rapid diagnosis avoids futile and often painful and ineffective intensive care to extend life in cases with grave prognosis, which helps to reduce the physical suffering of the patient and improves psycho-socio-economic issues in family members [9,10].

With compelling evidence on the diagnostic capacity and clinical utility of rWES and rWGS, national health care systems start to implement the technology as part of a routine clinical standard of care. The National Health Service (NHS) England in the United Kingdom (UK) launched the rWES service for critically ill children in October 2019 [11,12]. One of the major obstacles to universal clinical implementation is due to resource and budget constraints. Demonstration trials and clinical studies have primarily focused on the diagnostic capacity and speed of rWES and rWGS in critically ill infants younger than one year, evidence in older children beyond the intensive care setting is scarce [4,5,13,14]. Studies with economic impact of clinical management brought about by a rapid genetic diagnosis with detailed cost analyses were limited to only two studies, focusing in predominantly Caucasian and Hispanic/Latino children up to four years of age [5,6].

To date, the actual healthcare costs associated with rWES or rWGS has never been reported in the Asia Pacific Region. The current study sought to prospectively assess the diagnostic capacity, TAT, clinical utility, and the costs associated with precision medicine interventions of rWES in predominantly Chinese infants and children with suspected monogenic disorder beyond infancy, and in both intensive care units (ICUs) and non-ICUs.

Methods

Participants

Paediatric patients with suspected monogenic disorder were prospectively recruited from June 2016 to February 2020 by territory-wide referrals from paediatric and adolescent ICU and non-ICU settings to the clinical genetics service at the Queen Mary Hospital and the Hong Kong Children's Hospital (the University of Hong Kong affiliated hospitals) in Hong Kong. The recruitment criteria were broad with the following inclusion criteria: i) critically ill patients urging for a diagnosis; or ii) patients who would benefit from a timely diagnosis to support decision in clinical management. Multiple medical specialists including clinical geneticist, neonatologist, and paediatric subspecialists assessed all potential patients for inclusion eligibility. Patients with a clearly recognisable condition or syndrome where genetic testing offers no additional benefit, a known diagnosis, non-monogenic cause likely, and/or low urgency were excluded from this study. All eligible families were invited for rWES. Informed written consents were obtained from parents with pre-test counselling. Ethics approvals were granted by the Institutional Review Board, the University of Hong Kong/Hospital Authority Hong Kong West Cluster (UW12-211; UW18-520).

rWES, data analysis and interpretation

In this study, we aimed to provide a genetic diagnosis within a 14-day timeframe. The 14-day timeframe was chosen based on our best available resources, such as manpower and computing power, in the research setting. Trio-based rWES was performed whenever possible, where the major sources of DNA were peripheral blood and buccal mucosa. The Nextera DNA Exome Kit (Illumina) was used for library preparation. In most of the cases, we

Introduction

Genetic diseases and congenital malformations are the leading causes of prolonged hospitalisation and mortality in infants and children both internationally and in Hong Kong [1,2]. In the seven million-population in Hong Kong, one in 67 is living with at least one rare disease, with approximately 80% of the rare diseases being genetic in origin [3]. Despite the fact that rare genetic diseases are often life threatening or chronically debilitating, the rapid expansion and translational application of whole-exome sequencing (WES) and whole-genome sequencing (WGS) offer the potential to revolutionise the diagnostic trajectory of patients with medical complexity.

Since genetic diseases can progress quickly, a rapid genetic diagnosis is critical to reduce suffering, morbidity, and mortality, especially in acute clinical settings [4–6]. Rapid WES (rWES) and rapid WGS (rWGS) provide a much faster turnaround time (TAT) in days, as contrasted to a succession of different conventional diagnostic tests that may take several months to return, enabling diagnosis-predicated precision medicine and a shorter diagnostic

would wait for sample batching in order to save the costs. In other words, library preparation would begin when we received samples from two or more families indicated for rWES in the same batch of sequencing. The DNA libraries were sequenced using Illumina NextSeq500, with the aim to obtain an average depth of 100X for each sample. Reads alignment and variant calling were performed as described previously [15]. Variant analysis and interpretation were reviewed by multidisciplinary team members, including genome analyst, bioinformatician, clinical geneticist, neonatologist, and paediatric subspecialist as required. The pathogenicity of the variants was assessed using the American College of Medical Genetics and Genomics (ACMG) guideline [16]. High confident variants (variants with read depth $\geq 20X$ and $GQ \geq 20$) were communicated to the referring clinician before confirmation by sanger sequencing or other appropriate orthogonal methods.

Clinical utility and healthcare utilisation

Here, clinical utility is a measure of the relevance and usefulness of the molecular diagnosis brought about by rWES. The change in clinical management due to the genetic diagnosis was used as an indicator for clinical utility, and was classified into six major types of management according to Riggs et al. [17]: (i) referral to a specialist; (ii) further diagnostic testing indicated to evaluate possible complications; (iii) surgical or interventional procedures indicated or contraindicated; (iv) surveillance for potential complications associated with genetic variant; (v) medications indicated or contraindicated; and (vi) lifestyle changes. Management implication on genetic counselling about recurrence risk and family screening was not included because it was assumed to be applicable to all types of genetic test results.

The impact on clinical management was assessed in all diagnosed cases with quantitative changes in healthcare utilisation. In these cases, the actual healthcare utilisation was compared with a counterfactual diagnostic trajectory using either one of the two methods (whenever possible): (i) self-comparison; where planned clinical management was changed and/or avoided due to the rWES diagnosis; or (ii) case-control; where actual healthcare utilisation in the index case was compared with that of a matched historical control with the same molecular diagnosis that was made with available standard genetic tests and TAT. Actual healthcare utilisation data of the matched historical controls was obtained through the electronic patient record (ePR), and was discussed in an expert panel including the clinical geneticist, other medical subspecialist, genome analyst, bioinformatician, and genetic counsellor.

Economic impact of clinical management brought about by the rWES genetic diagnosis was estimated from the healthcare system perspective. Unit costs of investigations, procedures, medications, and hospital days were listed in the Supplementary Table 1. Costs were estimated using the 2018/19 unit costs provided by the Hospital Authority, the Government of the Hong Kong Special Administrative Region, and laboratory quotations [18,19]. For procedural costs that were not publicly available under the Hong Kong Hospital Authority setting, unit costs were obtained using the UK NHS national schedule of reference costs and set at 2018/19 prices [20]. The cost of rWES includes the cost of human exome library preparation, sequencing, reagents, data storage, server fees, sanger validation, labware, and labour. All costs in this study were reported in Hong Kong dollars (HKD), with an exchange rate of about 10·1 per British pound sterling (GBP) at the time of study.

Role of the funding source

The study was supported by the Health and Medical Research Fund (HMRF) by the Hong Kong Food and Health Bureau, the Seed

Fund for Basic Research by the University of Hong Kong, the Society for the Relief of Disabled Children, and the Edward and Yolanda Wong Fund. The funders of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding authors had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

Patient demographics

Between June 2016 to February 2020, 103 paediatric patients were eligible and invited for rWES. One family whose child had cardiac manifestation declined to participate in this study as the parents thought that genetic diagnosis would not impact clinical management. A total of 102 patients were enrolled into this study for data analysis. The characteristics of the recruited patients were summarized in Table 1. Demographic characteristics were reasonably well balanced with no statistical difference between the positive and negative cases. Among the 102 prospectively recruited patients, 46 (45%) were males and 56 (55%) were females. The median and mean age at time of enrolment was 174 days and 1,247 days (3·4 years), respectively, ranging from 1 day to 19·4 years of age [interquartile range (IQR): 40 – 2,005 days; standard deviation (SD): 1,830 days]. Consanguinity was uncommon ($n=2$, 2%). rWES was performed for 91 trios (89%), one quadruple (1%), five mother-infant duos (5%), and five singletons (5%).

A total of 46 patients (45%) were recruited from ICUs; of which 31 (30%) were from neonatal ICU (NICU) and 15 (15%) from paediatric ICU (PICU). Clinical presentations were highly diverse, and were classified according to the primary indication for testing (Table 1). The most common presentation for testing was neurological disorders ($n=24$; 24%), followed by cardiovascular disorders ($n=13$; 13%), and multiple congenital abnormalities ($n=12$; 12%).

Diagnostic performance and turnaround time

rWES achieved a molecular diagnosis in 32 of 102 patients, corresponding to a diagnostic yield of 31·4% (Table 2). Among the patients with a molecular diagnosis, 66% ($n=21$) had autosomal dominant (AD) disease; and 34% ($n=11$) had autosomal recessive (AR) disease. Among the patients with AD disease, 67% ($n=14$) had *de novo* mutations; whereas all patients with AR diseases inherited the mutations from their parents.

Seventeen of 46 patients (37%) recruited from ICUs received a molecular diagnosis (ten from NICU, seven from PICU). For non-ICUs inpatient, ten out of 38 (26%) received a molecular diagnosis; and five out of 18 outpatients (27%) were diagnosed molecularly. Diagnostic yield was also examined based on primary system involvement in each clinical setting (Supplementary Table 2).

The median and mean TAT of rWES from the date of sample retrieval for the entire cohort were 11 days and 12 days (IQR: 8 – 14; SD: 8·9), respectively. The median TAT of positive and negative cases was 11 (IQR: 8 – 12; SD: 13·9) and 12 days (IQR: 8 – 15; SD: 5·3), respectively. One outlier of 86 days was identified (RAP006). The mean TAT of the sampled cases was significantly shorter from the 14-day benchmark after removing the outlier ($p<0.0001$).

We were able to achieve the targeted 14 days of rapid TAT in 27 of 32 (84%) positive cases. The reasons for prolonged TAT included (i) restricted access to computing power due to bioinformatics server maintenance ($n=3$, RAP014, RAP036 and RAP106); (ii) laboratory closure due to renovation ($n=1$, RAP038); and (iii) international collaboration of case series ($n=1$, RAP006). In our cohort, RAP006 had the longest TAT of 86 days due to discussion

Table 1
Demographic characteristics of the 102 patients.

Characteristic		Overall (n=102)	Positive (n=32)	Negative (n=70)
Sex	Male	46 (45%)	15 (47%)	31 (44%)
	Female	56 (55%)	17 (53%)	39 (56%)
Referral source	NICU	31 (30%)	10 (31%)	21 (30%)
	PICU	15 (15%)	7 (22%)	8 (11%)
	Non-ICU inpatient	38 (38%)	10 (31%)	28 (40%)
	Genetics clinic / outpatient	18 (18%)	5 (16%)	13 (19%)
Age at recruitment	≤ 1 y	59 (58%)	22 (69%)	37 (53%)
	> 1 y	43 (42%)	10 (31%)	33 (47%)
	Median (range)	174 d (1d–19y)	137d (4d–19y)	199d (1d–17y)
Known parental consanguinity		2 (2%)	2 (6%)	0 (0%)
Primary system involved	Neurology	24 (24%)	9 (28%)	15 (21%)
	Cardiac	13 (13%)	3 (9%)	10 (14%)
	Multiple congenital abnormalities	12 (12%)	4 (13%)	8 (11%)
	Gastrointestinal	10 (10%)	3 (9%)	7 (10%)
	Haematology	10 (10%)	1 (3%)	9 (13%)
	Cancer	8 (8%)	3 (9%)	5 (7%)
	Endocrine	8 (8%)	2 (6%)	6 (9%)
	Respiratory	6 (6%)	1 (3%)	5 (7%)
	Immunology	5 (5%)	2 (6%)	3 (4%)
	Metabolic	2 (2%)	2 (6%)	0 (0%)
	Renal	2 (2%)	1 (3%)	1 (1%)
	Musculoskeletal	2 (2%)	1 (3%)	1 (1%)

ICU intensive care unit; NICU neonatal intensive care unit; PICU paediatric intensive care unit.

with international collaborators. Through the process, we identified five additional patients with the same phenotype carrying the same homozygous *COQ4* c.370G>A, allowing us to re-classify it from variant of unknown significance (VUS) to likely pathogenic. We have also shown that the c.370G>A variant is a founder mutation in Southern Chinese. This case enabled us to form the largest series of primary coenzyme Q₁₀ deficiency-7 (COQ10D7) reported in the literature [21].

Clinical utility and healthcare utilisation

Among the 32 positive diagnoses, rWES aided clinical management in 28 (88%). rWES most commonly aided management in the areas of surgical or interventional procedure indicated or contraindicated (n=11; 34%), followed by surveillance (n=10; 31%) and medication indicated or contraindicated (n=10; 31%), specialist referral (n=9; 28%), and further diagnostic testing (n=5; 16%) and life style changes (n=5; 16%) (Fig. 1). Detailed management change for each case was summarized in Supplementary Table 3.

The impact on changes in healthcare utilisation was quantified in eight patients (Table 3), in which five were estimated using the self-comparison approach, and three were compared with matched historical controls. The impact of clinical management could not be quantified in the remaining 20 of 28 patients without matched historical controls.

RAP014 was a three-month-old boy with persistent jaundice and conjugated hyperbilirubinaemia and deranged liver enzymes. Ultrasound scan and hepatobiliary scintigraphy was inconclusive for biliary atresia. Despite a subsequent Kasai operation and on-table cholangiogram, the child did not improve clinically and was put on waiting list for liver transplant. In view of the atypical course and findings, the child was offered rWES, which revealed a *de novo* *JAG1* mutation substantiating the genetic diagnosis of Alagille syndrome. As up to 90% Alagille syndrome patients would clinically improve without the need for transplant [22], liver transplant was withheld and medical treatment was optimized, and the child responded well. By achieving the rWES diagnosis within a TAT of 18 days, liver transplant was avoided, saving \$427,937.

RAP042 presented at 11 months old with recurrent episodes of hypoglycaemia, hepatomegaly, and neutropenia. The clinical picture was suspicious of glycogen storage disease (GSD) type Ib. By

conventional diagnostic pathway the child would have required a liver biopsy, or sanger sequencing which has a TAT of approximately eight weeks. During this time, the child would be empirically given granulocyte-macrophage colony stimulating factor (GM-CSF) for neutropenia. With rWES, the pathogenic variant of the *G6PC* gene was identified within seven days, making a molecular diagnosis of GSD type Ia, and showed no mutation in the gene of GSD type Ib. The accurate diagnosis rendered GM-CSF unnecessary. Costs of the GM-CSF treatment saved could not be compared due to the lack of a control case for direct comparison. However, avoidance of a liver biopsy as an alternative diagnostic modality alone saved \$22,230.

RAP068 was a neonate born to a mother with systemic lupus erythematosus on azathioprine, and presented shortly after birth with fetal bradycardia requiring cardiopulmonary resuscitation. Electrocardiogram (ECG) did not show any heart block, but blood tests revealed severe congenital pancytopenia, and multiple packed cells and platelet transfusions were given. Bone marrow aspiration and trephine biopsy were initially considered as work up for bone marrow failure, but rWES helped identify two variants in *NUDT15*. According to Clinical Pharmacogenetics Implementation Consortium guidelines the baby was classified as a poor metabolizer (*2/*3) of azathioprine, and the mother was an intermediate metabolizer (*1/*3) [23]. It is likely that with a standard dose, the azathioprine level in the fetal circulation was much elevated causing in-utero myelosuppression, and hence pancytopenia [24]. The rapid diagnosis revealed the status of drug metabolism and the baby spontaneously recovered with adjustments in maternal medication, breastfeeding abstinence and conservative management. Bone marrow aspiration and trephine biopsy were avoided, which saved \$56,075.

RAP118 was born with multiple congenital anomalies including craniofacial dysmorphism, right undescended testis and dynamic velopharyngeal obstruction requiring home oxygen. He also developed global developmental delay and failure to thrive during infancy. Previous karyotyping and chromosomal microarray did not reveal any abnormalities. He presented with recurrent episodes of abdominal distension, respiratory distress and pyrexia, as well as intractable epilepsy of unknown origin. Extensive workup including contrast studies did not identify a cause for the abdominal distension, and a full-thickness rectal biopsy was considered to rule

Table 2

All diagnoses (n=32) made by rWES in the cohort of 102 patients.

Patient	Sex	Age in days	Recruitment site	Disease category	Gene (Mode of inheritance)	Diagnosis (OMIM)	Nucleotide change(s)	Amino acid change(s)	Inheritance	TAT (days)
RAP005	M	1707	G/C	Cancer	<i>NF1</i> (AD)	Neurofibromatosis, type 1 (OMIM: 162200)	c.5839C>T	p.(Arg1947Ter)	<i>de novo</i>	7
RAP006	M	45	NICU	Metabolic	<i>COQ4</i> (AR)	Primary coenzyme Q10 deficiency-7 (OMIM: 616276)	c.[370G>A];[402+1G>C]	p.[(Gly124Ser)];[?]	Inherited	86
RAP009	F	3	NICU	Renal	<i>PKHD1</i> (AR)	Polycystic kidney disease 4 (OMIM: 263200)	c.[2107G>A];[9169delG]	p.[(Asp703Asn)]; [(Ala3057fs)]	Inherited	7
RAP014*	M	131	Non-ICU	Gastrointestinal	<i>JAG1</i> (AD)	Alagille syndrome 1 (OMIM: 118450)	c.2874_2875delTG	p.(Ala959fs)	<i>de novo</i>	18
RAP022*	M	4	NICU	Neurology	<i>ACTA1</i> (AD)	Nemaline myopathy (OMIM: 161800)	c.1001C>G	p.(Pro334Arg)	<i>de novo</i>	9
RAP026	M	43	NICU	Musculoskeletal	<i>COL1A1</i> (AD)	Osteogenesis imperfecta Type I-IV (OMIM: 166200, 166210, 259420, 166220)	c.3226G>A	p.(Gly1076Ser)	Inherited	8
RAP031	M	3779	Non-ICU	Endocrine	<i>SLC29A3</i> (AR)	Histiocytosis-lymphadenopathy plus syndrome (OMIM: 602782)	c.[1346C>G];[1346C>G]	p.[(Thr449Arg)]; [(Thr449Arg)]	Inherited	11
RAP036	F	127	PICU	Respiratory	<i>SOX10</i> (AD)	PCWH syndrome (Peripheral demyelinating neuropathy, central dysmyelinating leukodystrophy, Waardenburg syndrome, Hirschprung's disease) (OMIM: 609136)	c.1090C>T	p.(Gln364Ter)	<i>de novo</i>	15
RAP038*	F	9	NICU	Neurology	<i>KLHL40</i> (AR)	Autosomal-Recessive Nemaline Myopathy (OMIM: 615348)	c.[1516A>C]; [1516A>C]	p.[(Thr506Pro)]; [(Thr506Pro)]	Inherited	26
RAP042*	F	363	Non-ICU	Metabolic	<i>G6PC</i> (AR)	Glycogen storage disease Ia (OMIM: 232200)	c.[326G>A]; [1022T>A]	p.[(Cys109Tyr); [(Ile341Asn)]	Inherited	7
RAP043	F	318	Non-ICU	Neurology	<i>CTNNA1</i> (AD)	Neurodevelopmental disorder with spastic diplegia and visual defects (NEDSDV) (OMIM: 615075)	c.998dupA	p.(Tyr333Ter)	<i>de novo</i>	13
RAP045	M	46	NICU	MCA	<i>CHD7</i> (AD)	CHARGE syndrome (OMIM: 214800)	c.1510C>T	p.(Gln504Ter)	<i>de novo</i>	5
RAP047	F	7	Non-ICU	Cardiology	<i>TSC2</i> (AD)	Tuberous sclerosis-2 (OMIM: 613254)	c.4493+1G>A	p.?	<i>de novo</i>	12
RAP048	M	5467	Non-ICU	Cardiology	<i>RBM20</i> (AD)	Dilated cardiomyopathy-1DD (OMIM: 613172)	c.1906C>T	p.(Arg636Cys)	Inherited	6
RAP051*	F	1427	G/C	Immunology	<i>SERPING1</i> (AD)	Hereditary angioedema type 1 (OMIM: 106100)	c.1396C>T	p.(Arg466Cys)	Inherited	6
RAP053	M	137	Non-ICU	Cardiology	<i>TAB2</i> (AD)	Multiple types of congenital heart defects (OMIM: 614980)	c.1121dupC	p.(Asn375fs)	Inherited	11

(continued on next page)

Table 2 (continued)

Patient	Sex	Age in days	Recruitment site	Disease category	Gene (Mode of inheritance)	Diagnosis (OMIM)	Nucleotide change(s)	Amino acid change(s)	Inheritance	TAT (days)
RAP061	M	151	PICU	Neurology	SCO2 (AR)	Fatal Infantile Cardioencephalomyopathy (OMIM: 604377)	c.[210_229del]; [763C>T]	p.[(Leu71fs)]; [(Arg255Trp)]	Inherited	11
RAP064	F	34	NICU	Neurology	SCN2A (AD)	Early infantile epileptic encephalopathy-11 (OMIM: 613721)	c.4972C>T	p.(Pro1658Ser)	<i>de novo</i>	13
RAP066	F	7065	G/C	Neurology	SPG11 (AR)	Spastic paraplegia 11 (OMIM: 604360)	c.[5399_5402delinsTGGAGGAT]; [5399_5402delinsTGGAGGAT]	p.[(Gln1800fs)]; [(Gln1800fs)]	Inherited	11
RAP068 [^]	F	21	NICU	Haematology	NUDT15	{Thiopurines, poor metabolism of, 2} (OMIM: 616903)	c.[36_37insGGAGTC]; [415C>T]	p.[(Val18_Val19insGlyVal)]; [(Arg139Cys)]	Inherited	9
RAP075	M	160	Non-ICU	MCA	ARID1B (AD)	Coffin-Siris syndrome 1 (OMIM: 135900)	c.5394_5397del	p.(Phe1798fs)	<i>de novo</i>	11
RAP078	F	6241	PICU	Gastrointestinal	ATP7B (AR)	Wilson disease (OMIM: 277900)	c.[2333G>T]; [3155C>T]	p.[(Arg778Leu)]; [(Pro1052Leu)]	Inherited	10
RAP080	F	3328	Non-ICU	Gastrointestinal	ATP7B (AR)	Wilson disease (OMIM: 277900)	c.[1531C>T]; [1531C>T]	p.[(Gln511Ter)]; [(Gln511Ter)]	Inherited	9
RAP085	F	44	Non-ICU	Cancer	PTPN11 (AD)	Noonan syndrome 1 (OMIM: 163950)	c.1510A>G	p.(Met504Val)	<i>de novo</i>	12
RAP096	F	79	NICU	MCA	KMT2A (AD)	Wiedmann Steiner syndrome (OMIM: 605130)	c.9467T>A	p.(Leu3156Ter)	Not in mother	12
RAP098	M	5261	G/C	Neurology	ACTB (AD)	Juvenile-onset dystonia (OMIM: 607371)	c.547C>T	p.(Arg183Trp)	<i>de novo</i>	8
RAP102	M	32	NICU	Neurology	KCNQ2 (AD)	Spectrum of overlapping neonatal epileptic phenotypes, ranging from the milder form of seizures, benign neonatal, 1 (OMIM: 121200) to severe form of epileptic encephalopathy, early infantile, 7 (OMIM: 613720)	c.1025C>T	p.(Ser342Leu)	<i>de novo</i>	13
RAP106	F	1481	PICU	Neurology	GNAO1 (AD)	Epileptic encephalopathy, early infantile, 17 (OMIM: 615473) and neurodevelopmental disorder with involuntary movements (OMIM: 617493)	c.709G>A	p.(Glu237Lys)	<i>de novo</i>	15
RAP114	F	101	PICU	Cardiology	RAF1 (AD)	Noonan Syndrome 5 (OMIM: 611553)	c.784A>C	p.(Asn262His)	Inherited	11
RAP118 [*]	M	335	PICU	MCA	BRAF (AD)	Cardiofaciocutaneous syndrome (OMIM: 115150)	c.1785T>G	p.(Phe595Leu)	<i>de novo</i>	7
RAP119 [*]	F	3707	G/C	Endocrine	WNK4 (AD)	Pseudohypoadosteronism, type IIB (OMIM: 614491)	c.1685A>G	p.(Glu562Gly)	Inherited	11
RAP125	M	222	PICU	Immunology	IL7R (AR)	Severe combined immunodeficiency, T-cell/negative, B-cell/natural killer cell-positive type (OMIM: 608971)	c.[221+2T>A]; [361dupA]	p.[?];[(Ile121AsnfsTer8)]	Inherited	6

^{*}Healthcare cost estimation was performed for these cases. [^]This is a pharmacogenetic finding. As the patient's primary indication for rWES was to identify the cause of pancytopenia, and that the compound heterozygous variants in *NUDT15* with the history of maternal exposure to azathioprine during pregnancy could well explain the patient's phenotype. Therefore, this case was considered as a positive diagnosis. **AD** autosomal dominant; **AR** autosomal recessive; **F** female; **G/C** genetics clinic; **M** male; **MCA** multiple congenital abnormalities; **NICU** neonatal intensive care unit; **PICU** paediatric intensive care unit; **TAT** turnaround time

Table 3
Impact of rWES on changes in acute healthcare utilisation and associated healthcare costs in eight patients.

Patient	Clinical presentation	Gene (Diagnosis)	Expected management / outcome without diagnosis	Healthcare utilisation	Total costs (HKD)	Cost saved (HKD)
RAP014	Failed Kasai procedure with persistent cholestasis and liver function derangement	<i>JAG1</i> (Alagille syndrome 1)	Liver transplant	Optimised medical treatment; avoided liver transplant	-	\$427,937
RAP022	Fetal akinesia, generalised hypotonia	<i>ACTA1</i> (Nemaline myopathy)	Proceed to further invasive diagnostic tests; prolonged NICU stay with futile and ineffective treatment	Avoided EMG, muscle biopsy, NCV test; 47 NICU days (redirection of care at 47 days of life)	\$1,146,800	\$3,957,321
<i>RAP022_control</i>				EMG, muscle biopsy, NCV test; 205 NICU days (redirection of care at 205 days of life)	\$5,103,110	
RAP038	Fetal akinesia	<i>KLHL40</i> (AR-nemaline myopathy)	Progressive deterioration; proceed to further invasive diagnostic tests; prolonged NICU stay	Avoided EMG, muscle biopsy, sural nerve biopsy, NCV test; 61 NICU days (redirection of care at 61 days of life)	\$1,632,920	\$3,558,705
<i>RAP038_control</i>				EMG, muscle biopsy, sural nerve biopsy, NCV test; 342 IP days and 127 NICU days (passed away at 16 months old)	\$5,045,660	
RAP042	Recurrent hypoglycaemia, intermittent hepatomegaly, neutropenia	<i>G6PC</i> (GSD1a)	Continue to manage patient as GSD1b using GM-CSF	Avoided liver biopsy and the use of GM-CSF as treatment	-	\$22,230
RAP051	Recurrent angioedema	<i>SERPING1</i> (HAE type I)	Continue to manage angioedema with ineffective anti-allergic medications including anti-histamines and steroids	Prescribed anti-histamines and steroids for 32 days; C1 esterase inhibitor indicated in future events	\$54	\$6,139
<i>RAP051_control</i>				Continued treatment with antihistamines and steroids for 23 years with poor response	\$6,193	
RAP068	Severe congenital pancytopenia	<i>NUDT15</i> (THPM2)	Proceed to invasive diagnostic procedures for bone marrow failure	Avoided bone marrow aspiration and trephine biopsy	-	\$56,075
RAP118	Recurrent abdominal distension, severe failure to thrive	<i>BRAF</i> (CFC syndrome)	Proceed to full-thickness rectal biopsy to rule out Hirschsprung disease	Avoided full-thickness rectal biopsy	-	\$11,332
RAP119	Recurrent episodes of hyperkalaemia with poor control, elevated renin and aldosterone	<i>WNK4</i> (PHA type IIB)	Continue to manage patient as RTA type IV with lifelong ineffective medications including frusemide, fludrocortisone and sodium supplements	Prescription of hydrochlorothiazide; stopped frusemide, fludrocortisone, and sodium supplements	Annual cost of \$128	Annual cost of \$4,510
				Prior diagnosis: prescribed frusemide, fludrocortisone, and sodium supplements	Annual cost of \$4638	
Total healthcare savings					\$8,044,250 (GBPE796,460)	
Cost of rWES for 102 families					\$2,719,063 (GBPE269,214)	
Net healthcare savings					\$5,325,187 (GBPE527,246)	

AR autosomal recessive; CFC Cardiofaciocutaneous; EMG electromyogram; GM-CSF granulocyte-macrophage colony stimulating factor; GSD1a glycogen storage disorder type 1a; GSD1b glycogen storage disorder type 1b; HAE hereditary angioedema; HKD Hong Kong dollars; IP inpatient; NICU neonatal intensive care unit; NCV nerve conduction velocity; PHA Pseudohypoaldosteronism; RTA renal tubular acidosis; THPM2 Thiopurines, poor metabolism of, 2

Patient	Ward	Diagnosis (OMIM)	Gene	R	D	P	S	M	L	O
RAP005	Genetics clinic	Neurofibromatosis, type 1 (OMIM: 162200)	<i>NF1</i>							
RAP006	NICU	Primary coenzyme Q10 deficiency-7 (OMIM: 616276)	<i>COQ4</i>							
RAP009	NICU	Polycystic kidney disease 4 (OMIM: 263200)	<i>PKHD1</i>							
RAP014	Non-ICU	Alagille syndrome 1 (OMIM: 118450)	<i>JAG1</i>							
RAP022	NICU	Nemaline myopathy (OMIM: 161800)	<i>ACTA1</i>							
RAP026	NICU	Osteogenesis imperfecta Type I-IV (OMIM: 166200, 166210, 259420, 166220)	<i>COL1A1</i>							
RAP031	Non-ICU	Histiocytosis-lymphadenopathy plus syndrome (OMIM: 602782)	<i>SLC29A3</i>							
RAP036	PICU	PCWH syndrome (Peripheral demyelinating neuropathy, central dysmyelinating leukodystrophy, Waardenburg syndrome, Hirschprung's disease) (OMIM: 609136)	<i>SOX10</i>							
RAP038	NICU	Autosomal-Recessive Nemaline Myopathy (OMIM: 615348)	<i>KLHL40</i>							
RAP042	Non-ICU	Glycogen storage disease Ia (OMIM: 232200)	<i>G6PC</i>							
RAP043	Non-ICU	Neurodevelopmental disorder with spastic diplegia and visual defects (NEDSDV) (OMIM: 615075)	<i>CTNIB1</i>							
RAP045	NICU	CHARGE syndrome (OMIM: 214800)	<i>CHD7</i>							
RAP047	Non-ICU	Tuberous sclerosis-2 (OMIM: 613254)	<i>TSC2</i>							
RAP048	Non-ICU	Dilated cardiomyopathy-1DD (OMIM: 613172)	<i>RBM20</i>							
RAP051	Genetics clinic	Hereditary angioedema type 1 (OMIM: 106100)	<i>SERPING1</i>							
RAP053	Non-ICU	Multiple types of congenital heart defects (OMIM: 614980)	<i>TAB2</i>							
RAP061	PICU	Fatal Infantile Cardioencephalomyopathy (OMIM: 604377)	<i>SCO2</i>							
RAP064	NICU	Early infantile epileptic encephalopathy-11 (OMIM: 613721)	<i>SCN2A</i>							
RAP066	Genetics clinic	Spastic paraplegia 11 (OMIM: 604360)	<i>SPG11</i>							
RAP068	NICU	{Thiopurines, poor metabolism of, 2} (OMIM: 616903)	<i>NUDT15</i>							
RAP075	Non-ICU	Coffin-Siris syndrome 1 (OMIM: 135900)	<i>ARID1B</i>							
RAP078	PICU	Wilson disease (OMIM: 277900)	<i>ATP7B</i>							
RAP080	Non-ICU	Wilson disease (OMIM: 277900)	<i>ATP7B</i>							
RAP085	Non-ICU	Noonan syndrome 1 (OMIM: 163950)	<i>PTPN11</i>							
RAP096	NICU	Wiedmann Steiner syndrome (OMIM: 605130)	<i>KMT2A</i>							
RAP098	Genetics clinic	Juvenile-onset dystonia (OMIM: 607371)	<i>ACTB</i>							
RAP102	NICU	Spectrum of overlapping neonatal epileptic phenotypes, ranging from the milder form of seizures, benign neonatal, 1 (OMIM: 121200) to severe form of epileptic encephalopathy, early infantile, 7 (OMIM: 613720)	<i>KCNQ2</i>							
RAP106	PICU	Epileptic encephalopathy, early infantile, 17 (OMIM: 615473) and neurodevelopmental disorder with involuntary movements (OMIM: 617493)	<i>GNAO1</i>							
RAP114	PICU	Noonan Syndrome 5 (OMIM: 611553)	<i>RAF1</i>							
RAP118	PICU	Noonan Syndrome 7 (OMIM: 164757)	<i>BRAF</i>							
RAP119	Genetics clinic	Pseudohypoaldosteronism, type IIB (OMIM: 614491)	<i>WNK4</i>							
RAP125	PICU	Severe combined immunodeficiency, T-cell/negative, B-cell/natural killer cell-positive type (OMIM: 608971)	<i>IL7R</i>							
Total (%)				9 (28)	5 (16)	11 (34)	10 (31)	10 (31)	5 (16)	2 (6)

Fig. 1. Impact on clinical management in 32 diagnosed patients.

D Diagnostic testing; **L** Lifestyle changes; **M** Medication; **O** Others; **P** Procedure; **R** Referral; **S** Surveillance

*A detailed version with specific changes in clinical management is available in Supplementary Table 3

out Hirschsprung disease. rWES revealed a *de novo* pathogenic mutation in *BRAF* gene which substantiated the diagnosis of cardiofaciocutaneous (CFC) syndrome. With no other suspicious gastrointestinal features, functional megacolon was considered as a complication of CFC syndrome [25]. Therefore, the invasive rectal biopsy was no longer required, saving \$11,332 and avoiding the psychological stress for the child.

RAP119 had recurrent episodes of hyperkalaemia of up to 7.4mEq/L since infancy. She was provisionally managed as renal tubular acidosis (RTA) type IV. Although the electrolyte levels were reasonably controlled over the years, a definite diagnosis was never reached despite extensive endocrine investigations. Episodes of electrolyte disturbances lead to repeated hospital admissions. At the age of 10, she was referred to the clinical genetics, and rWES identified a mutation in *WNK4* which led to the diagnosis of pseudohypoaldosteronism (PHA) type IIB. Hydrochlorothiazide was the treatment drug of choice for this condition [26], while her previous prescriptions of frusemide, fludrocortisone and sodium supplements were not effective treatments. The diagnosis allowed a switch to effective targeted therapy. Although it is difficult to estimate the lifelong costs saved for this patient, a minimum annual cost of \$4,510 was saved based on the child's previous prescription history.

RAP022 was born with fetal akinesia and was immediately transferred to NICU for ventilator support from birth. Initial assessment showed generalized hypotonia and areflexia with paucity of spontaneous limb movements. Extensive neurological diagnostic tests including electromyogram (EMG), nerve conduction velocity (NCV) and muscle biopsy under general anaesthesia were planned for. He was enrolled for rWES on the 5th day of life, and a *de novo* mutation of *ACTA1* was identified in nine days, leading to the diagnosis of a severe congenital type of nemaline my-

opathy. With the molecular diagnosis, the neurological diagnostic tests were deemed unnecessary. In view of the grave prognosis, the family opted for redirection of care and active withdrawal of treatment, and the baby passed away on the 47th day of life. In comparison, a matched control newborn was admitted to the same NICU five years earlier with the same clinical presentation, and extensive investigations including EMG, NCV and muscle biopsies did not reach a conclusive diagnosis. Eventually, a palliative approach was adopted in view of guarded prognosis, and the baby passed away on the 205th day of life. Post-mortem genetic studies were performed in an overseas laboratory three years later and eventually identified a positive *ACTA1* mutation associated with nemaline myopathy. Compared to the first case (RAP022), this case stayed in the NICU for an additional 158 days. rWES for RAP022 saved a total of \$102,121 procedural cost and \$3,855,200 inpatient cost in NICU.

Similarly, RAP038 was admitted to NICU at birth with fetal akinesia and ventilator dependency. rWES revealed mutation in *KLHL40* and a diagnosis of AR nemaline myopathy, aiding redirection to palliative care on the 61st day of life. In comparison, a control case with the same clinical presentation was admitted to the same NICU three years earlier, and had undergone a series of extensive investigations including NCV, EMG, muscle biopsy and sural nerve biopsy, before reaching a diagnosis of nemaline myopathy. The control case stayed in the NICU for 127 days and paediatric ward for 342 days before eventually passing away at 16 months old. The rWES avoided the need for a series of investigations and reduced the length of inpatient stay in NICU and paediatric ward for RAP038, saving a total of \$3,558,705.

RAP051 was referred to clinical genetic services at three years of age, with recurrent unprovoked attacks of angioedema since the age of one, with increasing frequency with age. She had

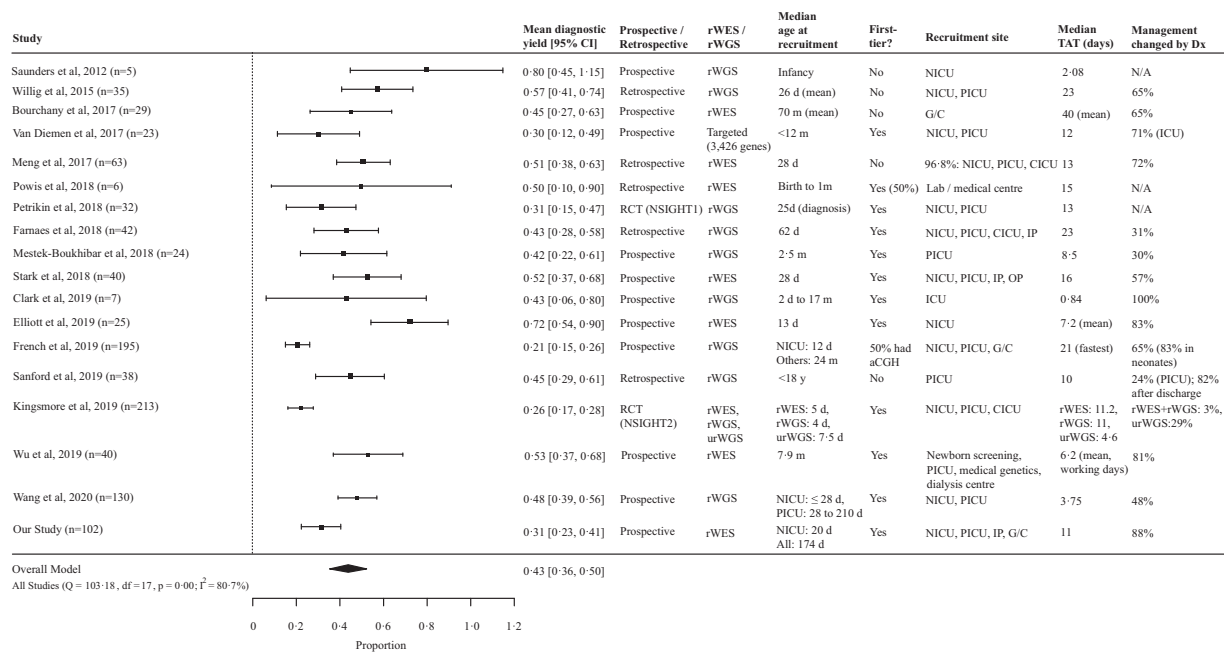


Fig. 2. Comparing this study with other published rWES and rWGS studies.

aCGH array comparative genomic hybridization; **CI** confidence interval; **CICU** cardiovascular intensive care unit; **Dx** diagnosis; **G/C** genetics clinic; **IP** inpatient; **N/A** not available; **NICU** neonatal intensive care unit; **OP** outpatient; **PICU** paediatric intensive care unit; **RCT** randomised controlled trial; **rWGS** rapid whole-genome sequencing; **rWES** rapid whole-exome sequencing; **TAT** turnaround time; **urWGS** ultra-rapid whole-genome sequencing

all along been managed with conventional treatment including antihistamines and salbutamol inhalers, with only fair effectiveness. rWES was offered and identified a mutation in *SERPING1* which led to the diagnosis of hereditary angioedema (HAE) type 1 within six days. HAE-related angioedema typically spontaneously resolves within two to five days, although severe cases can be life-threatening and require life-saving targeted medications. HAE angioedema episodes are well treated and prevented by C1 esterase inhibitors, but are unresponsive to conventional treatment including antihistamines, steroids and catecholamines [27,28]. The rWES diagnosis solved the diagnostic mystery and allowed a switch to effective drugs to control future attacks. In contrast, the control case presented in year 1996 at the age of 22 with recurrent episodes of angioedema and shortness of breath. Over 23 years, he had 11 documented visits to the Accident & Emergency Department for the same complaints, five of which required inpatient admission and management for at least three days for severe symptoms. Multiple antihistamines and steroids were given to control the symptoms each time, but with only limited response. He was finally referred to Clinical Immunology in 2018, and sanger sequencing revealed the diagnosis of HAE type 1. He had been given medication with no effect for 23 years, before being correctly prescribed C1 esterase inhibitor. For RAP051, the rWES diagnosis prevented the use of ineffective medications for the additional duration seen in the control case, saving \$6,139. rWES improved the care for patients by impacting their clinical management. In these eight patients where changes in acute healthcare utilisation were quantifiable, rWES was estimated to reduce the total hospital length of stay by 566 days and decrease the total healthcare costs by at least \$8,044,250 (GBP£796,460). The full cost of rWES was estimated to be \$9,343.9 per sample. The total cost of rWES for 102 families (291 samples: six singletons, four duos, 91 trios, one quadruple) was \$2,719,063 (GBP£269,214), much less than the total cost-savings in only eight patients. The net total healthcare cost-savings for the whole cohort was estimated to be \$5,325,187 (GBP £527,246).

Discussion

The study demonstrated the impact of rWES on clinical management and healthcare utilisation in a rapid manner in 102 predominantly Chinese infants and children with suspected genetic disorders. To date, this is the largest cohort with detailed healthcare cost implications associated with clinical management, and the fourth largest rWES / rWGS study in literature. The three studies with a sample size larger than 100 were all published recently (2019–2020). Despite a wide age range, broad spectrum of clinical presentations, and recruitment from various clinical settings, the diagnostic yield of 31.4% in this study was comparable to international studies. A systematic review of all rWES and rWGS publications from 2012 to 2020 was performed along with our findings (Fig. 2) [4–6,8,10,13,14,29–38]. The full search strategy was included in the supplementary file. In summary, in 18 studies comprising 1,049 patients with diverse clinical presentations, the pooled diagnostic yield was found to be 43% (95% CI 36%–50%, I² = 80.7%) using a random-effects model, ranging from 21% (n=195) to 80% (n=5). Such large difference might be explained by considerable between-study heterogeneity. It was found that the diagnostic rate was negatively correlated with the sample size (r²=0.36, p=0.009) (Supplementary Figure 1). There was no significant difference in diagnostic yield when comparing the use of rWES and rWGS (p>0.05), further supporting the findings from the randomised control trial by Kingsmore et al. [37] (2019), and the conclusion made in a meta-analysis of diagnostic utility made by standard WES and WGS [39]. With a lower unit cost and a similar diagnostic capacity of WES compared with that of WGS, it is reasonable to use WES in clinical practice. Severe heterogeneity in reporting TAT, age at recruitment, and disease type precluded statistical comparisons. Nevertheless, our median TAT of 11 days was comparable to international rWES and rWGS studies (Fig. 2). TAT was reported to be as short as a median of 20.17 h and 50 h, demonstrated in a cohort of seven ICU patients by using automated phenotyping and interpretation [34], and in a proof-of-

concept cohort of five NICU patients [10], respectively. It would be challenging however, to achieve such a short median TAT in a large-scale clinical setting. Achieving the best achieved TAT in this study (three days) sustainably would require a 24-7 sample processing and data analysis approach, dynamic sequencing of individual sample independent of sample batching, and a dedicated computing server for bioinformatics [5]. It would also significantly increase the cost for rWES. In fact, French et al. [4] demonstrated that a TAT of two to three weeks was adequate to impact most clinical decision making for patients suspected with single gene disorder within the NHS. Automated pipeline might be the new trend to achieve rapid TAT sustainably, though would require to be adapted for use in different healthcare systems.

Prior to the next generation sequencing (NGS) era, prolonged waiting time for receiving a genetic diagnosis is common in clinical genetics, it could take months or even years to uncover the underlying cause, making it impractical to facilitate urgent management decisions. In addition to the rapid TAT from date of sample retrieval to date of reporting to clinician, rWES is powerful in shortening the often stressful and exhausting diagnostic trajectory in patients. We have identified a girl with Alagille syndrome (*JAG1*) who was diagnosed using conventional genetic panel testing at the age of 20. She was presented with persistent cholestasis and gradual impairment of liver function at birth, and was extensively worked up for metabolic liver disease and connective tissue disorders over the years. Results from the conventional genetic test took six months to return, and the patient's diagnostic odyssey spanned over 20 years, contrasting to a TAT of 18 days and a diagnostic odyssey of five months in RAP014 diagnosed using rWES. The speed of rWES was also demonstrated in the case of RAP051 and its matched historical control, in which they were diagnosed with HAE type 1 (*SERPING1*) using rWES and conventional sanger sequencing respectively (TAT of six days and a three-year diagnostic journey vs. TAT of three months and a 23-year diagnostic journey). The rapid diagnosis ends the years of uncertainty and improves psychological wellbeing for the patient. Among the 32 diagnoses made by rWES, conventional genetic tests are not available for nine of them from local clinical molecular diagnostic services, making it impossible to make the genetic diagnosis unless WES is available [40]. For those where standard genetic testing is available, the prolonged TAT of approximately four months makes it impractical to facilitate clinical management in urgent cases. rWES influenced early clinical management in 88% of diagnosed patients in this cohort, higher than most of the published studies. The percentage could be increased to 100% if genetic counselling about recurrence risk and family screening were also included. Some of the changes in clinical management captured in this study were similarly demonstrated in the study by Farnaes et al. [6], such as in the cases of Alagille syndrome (*JAG1*) and nemaline myopathy (*ACTA1* in our cohort, *NEB* in Farnaes' cohort), showing the reliability of management implications. In fact, rWES-based precision medicine may not be limited to the positively diagnosed patients, but also applicable to VUS cases and families with mild phenotypes. RAP123 was offered rWES for the suspicion of cerebral phaeohyphomycosis suspected to be caused by *Alternaria* spp. The compound heterozygous mutations in *CARD9* have not been reported and were classified as VUS. This promoted caution on prolonging duration of anti-fungal therapy as well as further functional study of the VUS to guide future management, as fungal infection is a signature of *CARD9* mutation. In diseases with incomplete penetrance and variable expressivity, rWES may help to uncover asymptomatic disease carriers in the parents in addition to revealing the diagnosis in the proband, prompting clinical management in the family level, such as in the cases of HAE (RAP051) and PHA type IIB (RAP119). The diagnosis of AD inherited HAE type 1 uncovered the missed case of the patient's mother, which led

to indication of self-initiated home treatment with plasma derived or recombinant C1 esterase inhibitor to abort attack and prevent hospitalisation. The diagnosis of PHA type IIB in RAP119 prompted blood check for the father and revealed that the father was also hyperkalaemic, in which he required immediate hospital admission and treatment. It also helped to uncover PHA type IIB in the paternal aunt and paternal grandmother.

While rWES is an expensive test, costs associated with unnecessary hospitalisation, management procedures, and treatment medications produced large cost-savings. Although the economic impact of rWES is not widely studied in this context, different approaches were adopted to quantify and estimate the associated costs. In a recent study by Wang et al. (2020) in China [38], optimised trio-based genome sequencing was reported to reduce medical care costs in 93% (14/15) of the patients, though none of the cost comparison was demonstrated. In the United States, Farnaes et al. (2018) estimated the cost-savings by comparing actual utilisation with "counterfactual utilisation" and/or matched historical controls [6]. In Australia, Stark et al. (2018) performed a cost-effectiveness analysis to compare the clinical- and cost-effectiveness of rWES with that of a previous cohort in 2014-15 that compared standard WES and conventional diagnostic methods [5,41]. Although the impact of precision medicine was only quantified in eight patients in this study, the avoided healthcare care costs (HKD\$8,044,250; GBP£796,460) far exceeded the costs of providing rWES for the entire cohort (HKD\$2,719,063; GBP£269,214). This was also in-line with the studies by Farnaes et al. [6] (2018) and Stark et al. [5] (2018), in which costs associated with changes in management were estimated in six patients in both cohorts, reducing healthcare costs by USD\$803,199 (HKD\$6,264,952) and by AU\$543,178 (HKD\$2,715,890), respectively. Most cost-savings were seen in the decision of palliative care, which was also the case in Farnaes et al.'s (2018) study [6]. This was due to the extremely expensive cost per day in intensive care unit. Decision in redirection of care is often difficult for families; a rapid genetic diagnosis gives confidence to families to avoid further suffering of the child with poor prognosis, and significantly reduces costs associated with healthcare utilisation and hospitalisation. Healthcare cost-savings in this study were considered to be underestimated as decreased utilisation of resources and hospitalisation were not accounted in cases without matched historical controls and/or without clear quantifiable documentation. In addition, intangible benefits were not captured in the study, such as family cascade testing, reproductive planning, management implications in VUS cases, and possible early detection of problems from long-term surveillance. These are important and invaluable for patients, families, and healthcare providers. Further reduction in healthcare utilisation and cost-savings would be expected in a longer term in cases with lifelong medications and repeated hospital readmissions. Though the impact on clinical outcomes for some cases was obvious, it was difficult to evaluate without extensive longitudinal follow-up.

In this study, four patients did not have direct clinical management implication as a result of a positive diagnosis from rWES. For example, mutations in *TAB2* (RAP053) and *KMT2A* (RAP096) were relatively new genetic diseases and hence little was known about the management and prognosis. However, the advancement of NGS technologies has significantly aided the discovery and delineation of new genetic diseases and we believe that the natural history and management guideline for these newly discovered diseases would soon be available. In fact, the *COQ4* mutations identified in RAP006 had led us to the discovery of ten additional patients sharing the same Southern Chinese-specific founder mutation [21]. Our case series suggested that neonatal onset of *COQ10D7* has a more severe clinical presentation and supplement treatment of *CoQ10* is not effective. Therefore if we encountered another neonatal patient with *COQ4* mutations, genetic diagnosis might inform redirection

of care, avoiding further suffering of the patient and reducing NICU costs.

We used the UK NHS system as a comparison to project service use pattern for implementation in the Hong Kong public healthcare system, as the Hong Kong Hospital Authority and the UK NHS share quite a lot of similarities. Up till January 2020, the NHS England has offered rWES to 80 children since the introduction of the service in October 2019 (27 children per month), and was expected to offer to up to 700 children annually [11,12]. With an approximate 10 times less paediatric population (0–19 years) in Hong Kong compared to that in England (1,149,800 vs. 13,241,287 in mid-2018) [42,43], our study showed that rWES was offered to approximately two to three children per month across the study period, showing a similar rate of service need for rWES as the NHS England. Although we received territory wider referrals across various clinical settings, rWES is not offered under the Hospital Authority at the moment. The number of patients requiring this service is expected to increase when it becomes available as part of the routine clinical care. It would be reasonable to offer rWES to 70 children per year in Hong Kong if this extends to be a territory wide service. When considering the implementation of rWES service as part of the routine clinical care in the Hong Kong public healthcare system, it is recommended to refer to the practice in the NHS.

Several limitations were acknowledged in this study. Firstly, similar to other clinical genetic studies, the results of this study relied on the clinical judgement of clinicians at every step, starting from patient recruitment. Since different clinicians may have variable level of understanding in rare disease genetics, we recommended a multidisciplinary team approach that involves the clinical geneticist in every step to provide expertise in rare disease genetics and to ensure patients recruited would benefit from the rWES [44]. In fact, this multidisciplinary team approach has been widely adapted in rare disease genomics. Secondly, a complete cost-effectiveness study including all costs of diagnostic investigations and procedures was not performed, though the cohort was well suited to assess the clinical utility and healthcare utilisation rather than to compare rWES over standard WES or conventional methods. The cost-effectiveness of standard WES and WGS in outpatient and inpatient settings has been demonstrated in previous studies, with particular focus on the diagnostic trajectory prior to genetic diagnosis [41,45,46]. Parallel comparison of rWES and conventional methods is more challenging, as the situation is more urgent and critical, and immediate clinical management decision should be made to improve patient's outcome. This precludes a comprehensive parallel comparison with conventional diagnostic methods, and therefore, to the best of our knowledge, cost-effectiveness analysis of rWES in a "self-comparison" manner is still lacking in literature. Thirdly, while the current study shows large healthcare cost-savings, it was based on eight cases and three matched historical controls; finding another case with the same molecular diagnosis made by conventional methods is difficult because genetic diseases are heterogeneous and are individually rare. The net healthcare cost-savings is expected to increase if the remaining 20 cases with impact on clinical management could be quantified. Lastly, cost-savings were estimated based on acute precision medicine interventions for the diagnosed patient, without taking account into the longer-term costs associated with the management. Extensive longitudinal follow-up of these families should be evaluated to provide clinical and economic evidence from the societal perspective.

Conclusion

This study illustrates that rWES in the paediatric and adolescent clinical setting in Hong Kong is feasible, has high diagnostic and clinical utility, reduces healthcare utilisation costs, and is compara-

ble to international standards. The magnitude and types of impact of rWES were corroborated by data from North American, European, Australian, and other Asian cohorts, combined as part of a meta-analysis, and were replicated in this predominantly Chinese population from the Asia Pacific region. Our study shed light on the possibility of using rWES to modify the treatment for patients with urgent needs in the clinical setting.

Contributors

CCYC, GKCL, CCYM, KSY, and BHYC contributed to the conception and design of the study. ML, SLCP, and JFTC performed the molecular laboratory work. GKCL, CCYM, JLFF, MHYC, JFTC, MCYC, MHYT, and KSY analysed the whole exome sequencing data. CCYC, GKCL, CCYM, JLFF, and JCKC evaluated the data on clinical management. CCYC, GKCL, and WHSW performed the statistical analyses. CCYC performed the cost analysis and involved in data organisation and presentation. CCYC and VCCH conducted the systematic review. JYLT, KSL, YKN, CWF, MSCW, RMSW, YLL, GCFC, SLL, and BHYC were the core members of the clinical review panel. CCYC and GKCL drafted the manuscript. CCYM, JLFF, JCKC, YLL, GCFC, SLL, KSY, and BHYC critically reviewed the manuscript with suggestions for improvement and revision. KSY and BHYC oversaw and supervised the project. All authors contributed to the overall data interpretation, reviewed, and approved the final draft for submission.

Declaration of Competing Interest

The authors declare that they have no competing interests.

Acknowledgments

We would like to thank the medical staff from territory-wide paediatric and adolescent clinical settings for the referrals, the healthcare professionals who managed the patients, and the patients and their families for their participation in the study. This study was supported by the Health and Medical Research Fund (HMRF) by the Hong Kong Food and Health Bureau, the Seed Fund for Basic Research by the University of Hong Kong, the Society for the Relief of Disabled Children, and the Edward and Yolanda Wong Fund. The views expressed are those of the authors and not necessarily those of the funders.

Data sharing

Study protocol, informed consent form, and individual participant data that underlie the results reported in this article, after de-identification, are available to investigators whose proposed use of the data has been approved by an independent review committee. Data will be available from the corresponding authors up to five years following publication.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at [doi:10.1016/j.lanwpc.2020.100001](https://doi.org/10.1016/j.lanwpc.2020.100001).

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