

# Expanding the genotypes and phenotypes for 19 rare diseases by exome sequencing performed in pediatric intensive care unit

Juan Liu<sup>1</sup> | Yu Zheng<sup>2,3</sup>  | Jiaotian Huang<sup>1</sup> | Desheng Zhu<sup>1</sup> | Ping Zang<sup>1</sup> | Zhenqing Luo<sup>2</sup> | Yongjia Yang<sup>2</sup>  | Yu Peng<sup>2</sup> | Zhenghui Xiao<sup>1</sup> | Yimin Zhu<sup>4</sup> | Xiulan Lu<sup>1</sup> 

<sup>1</sup>Pediatric Intensive Care Unit, Hunan Childrens Hospital, University of South China, Changsha, Hunan, China

<sup>2</sup>Pediatrics Research Institute of Hunan Province, Hunan Children's Hospital, Changsha, Hunan, China

<sup>3</sup>Center for Medical Genetics, School of Life Sciences, Central South University, Changsha, Hunan, China

<sup>4</sup>Emergency Medicine Institute of Hunan Province, Changsha, Hunan, China

## Correspondence

Zhenghui Xiao and Xiulan Lu, #86 Ziyuan Rd, Changsha, 410007 Hunan, China.  
Email: xiaozh888@126.com and 391118947@qq.com

Yimin Zhu, #61 Jiefang Rd, Changsha, 410005 Hunan, China.

Email: cszhuyimin@163.com

## Funding information

the Key Laboratory of Emergency Medicine for Children, Grant/Award Number: 2018TP1028; the Ministry of Science and Technology in China, Grant/Award Number: 2012BAI04B02

## Abstract

Phenotypes of some rare genetic diseases are atypical and it is a challenge for pediatric intensive care units (PICUs) to diagnose and manage such patients in an emergency. In this study, we investigated 58 PICU patients (39 deceased and 19 surviving) in critical ill status or died shortly without a clear etiology. Whole exome sequencing was performed of 103 DNA samples from their families. Disease-causing single-nucleotide variants (SNVs) and copy number variants (CNVs) were identified to do genotype-phenotypes analysis. In total, 27 (46.6%) patients received a genetic diagnosis. We identified 34 pathogenic or likely pathogenic SNVs from 26 genes, which are related to at least 19 rare diseases. Each rare disease involved an isolated patient except two patients caused by the same gene *ACAT1*. The genotypic spectrum was expanded by 23 novel SNVs from gene *MARS1*, *PRRT2*, *TBCK*, *TOR1A*, *ECE1*, *ARX*, *ZEB2*, *ACAT1*, *CPS1*, *VWF*, *NBAS*, *COG4*, and *INVS*. We also identified two novel pathogenic CNVs. Phenotypes associated with respiratory, multiple congenital anomalies, neuromuscular, or metabolic disorders were the most common. Twenty patients (74.1%) accompanied severe infection, 19 patients (70.1%) died. In summary, our findings expanded the genotypes and phenotypes of 19 rare diseases from PICU with complex characteristics.

## KEYWORDS

exome sequencing (ES), genetic disease, pediatric intensive care unit (PICU), phenotype, variant

## 1 | INTRODUCTION

Critical diseases caused by undiagnosed genetic conditions are challenging for pediatric intensive care units (PICUs). For most PICU patients, the symptoms are usually complicated by emergency admission, and some of them are in critical condition with unexplained liver failure, renal trauma,

or consciousness disturbances. It is now known that genetic disorders (congenital defects) are the major reasons for hospitalization and mortality in infants in neonatal intensive care units (NICUs) or PICUs (Weiner et al., 2011). In the past 6 years, by using exome sequencing (ES) or whole-genome sequencing (WGS) in 1185 children in PICUs/NICUs, the diagnostic rate of genetic disorders reached about 19%–69.7% globally

Juan Liu and Yu Zheng contributed equally to this study as co-first authors.

This is an open access article under the terms of the Creative Commons Attribution-NonCommercial License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited and is not used for commercial purposes.

© 2021 The Authors. *Human Mutation* published by Wiley Periodicals LLC

(Australian Genomics Health Alliance Acute Care Flagship, 2020; Clark et al., 2019; Daoud et al., 2016; Elliott et al., 2019; Farnaes et al., 2018; French et al., 2019; Gubbels et al., 2020; James et al., 2020; Kingsmore et al., 2019; Kingsmore et al., 2020; Meng et al., 2017; Petrikin et al., 2018; Ritter et al., 2020; Sanford et al., 2019; Schofield et al., 2019; L. D. Smith et al., 2015; Soden et al., 2014; Stark et al., 2017, 2019, 2016; N. B. Tan et al., 2019; T. Y. Tan et al., 2017; van Diemen et al., 2017; H. Wang, Lu, et al., 2020; H. Wang, Qian, et al., 2020; Willig et al., 2015; Wu et al., 2019). This dramatically improved the diagnosis, treatment, and prognosis in clinical work (Arts et al., 2019; Australian Genomics Health Alliance Acute Care Flagship, 2020; Farnaes et al., 2018; French et al., 2019; Meng et al., 2017; Mestek-Boukhibar et al., 2018; Ritter et al., 2020; Sanford et al., 2019; Schofield et al., 2019; Soden et al., 2014; Stark et al., 2017; Stark et al., 2019; Z. A.-O. Stark et al.; N. B. Tan et al., 2019; T. Y. Tan et al., 2017; H. Wang, Lu, et al., 2020; H. Wang, Qian, et al., 2020; Willig et al., 2015; Wu et al., 2019).

In western developed countries, it was estimated that more than 40 million children have rare genetic disorders, and the mortality rate reached about 30% in patients under 5 years old (Kuehne et al., 2019). In developing countries, with the gradual popularization of next-generation sequencing technology, rapid detection of genetic disorders in PICU patients has become possible. In the developed area of China, three studies from the Children's Hospital of Fudan University in Shanghai revealed that 85 of 163 pediatric patients had genetic findings (H. Wang, Lu, et al., 2020; H. Wang, Qian, et al., 2020), and 44 in 223 deceased newborns received genetic diagnosis (Yang et al., 2020). However, many rare diseases have atypical phenotypes. French et al. (2019) stated that 90% of the molecular diagnosis-positive patients had phenotypic descriptions that were atypical, and about 10% of the patients had a genetic diagnosis whose phenotype precisely predicted underlying genes. In the central south of China, before the clinical ES, WGS, and CNV analysis were available, patients usually had a long turn-around time in different hospitals with more complex clinic features. This increases the difficulty for PICU clinicians to diagnose precisely and manage shortly. Therefore, this study was aimed to identify the primary disease-causing factors underlying the complex and severe symptoms from PICU, and thus to obtain clues for precise diagnosis and improvement of clinical management of some rare genetic diseases.

In this study, we retrospectively investigated the deceased or critically ill children without clear etiology in one central PICU by WES. By screening single-nucleotide variants (SNVs), short insertions or deletions (InDels), and copy number variants (CNVs) simultaneously, we then identified disease-causing variants and further investigated the genotypes and phenotypes of the patients with genetic findings.

## 2 | MATERIALS AND METHODS

### 2.1 | Clinical patient selection

Hunan Children's Hospital is a central hospital in pediatrics in Hunan province. From September 20, 2014 to December 30, 2018, 5795 patients were transferred to the PICU department from other hospitals

in the local prefectural area. Initially, we recruited the PICU patients who were in dying status or without good reactions to regular therapies and who suspected a genetic disorder but had not undergone any genetic testing. Finally, after excluding patients with known etiology or rejected by parents or guardians, 58 patients participated having at least one of the following conditions: (1) high risk of short-term mortality but lacking medical explanation; (2) severe unexplained seizures or hypotonia; (3) metabolic crisis of unknown cause; (4) immunodeficiency of unknown origin; (4) multiple system failures of unknown pathogenesis; (5) multiple congenital anomalies or abnormal development; and (6) other uncertain etiologies (Borghesi et al., 2017). Figure 1 shows the flow diagram of the selection of the studied patients.

The investigated patients included 33 males and 25 females with the age between one month and 15 years old. The average age was 2.2 years old. Appropriate informed consent was obtained from the parents/guardians of all participants before sample collection. We collected detailed information on disease history, family history, and clinical features of each patient. Phenotypes were standardized using human phenotype ontology (HPO) (Köhler et al., 2017) and the Chinese Human Phenotype Ontology Consortium.

### 2.2 | Sample collection

Peripheral blood (2–3 ml) was collected from each participant using edetate disodium, including 23 trios, 28 probands only, three families (proband + parents + siblings), and four paired WES (proband + father/mother).

In total, we collected 117 samples from 58 patients and 30 family controls, and the genomic DNA of each sample was extracted using the standard phenol-chloroform protocol.

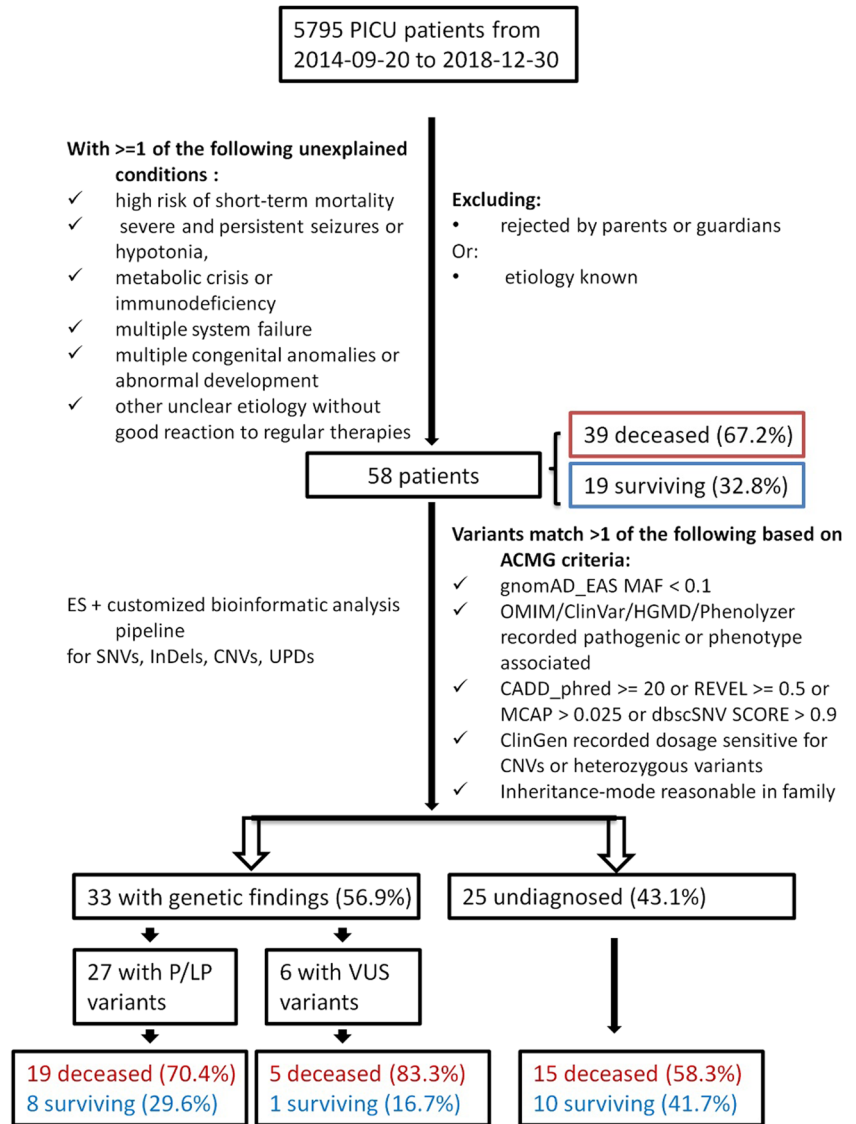
### 2.3 | Exome sequencing

One  $\mu$ g genomic DNA samples were fragmented to an average size of 180–280 bp and subjected to exome capture using the Agilent SureSelect Human All Exon V6 Kit (Agilent Technologies) according to the manufacturer's instructions. The prepared libraries were then sequenced with  $2 \times 150$  bp reads using the Illumina NovaSeq. 6000 system (Illumina) following the manufacturer's instructions. Each sample yielded more than 10 GB of raw data with more than 90% bases having a Phred quality score  $> 30$ . The mean coverage was  $\times 100$  of the genome and the minimum coverage of  $\times 10$  was about 99%.

### 2.4 | Bioinformatic analysis and interpretation

The raw reads were preprocessed using trim\_galore (version 0.6.4, [http://www.bioinformatics.babraham.ac.uk/projects/trim\\_galore/](http://www.bioinformatics.babraham.ac.uk/projects/trim_galore/)) to remove adapter-contaminated ends and low-quality bases with Phred scores  $< 20$ . The polished reads with a length  $\geq 80$  bp were subsequently mapped to the human reference genome (version: GRCh38) using

**FIGURE 1** Flow diagram of patients' selection and genetic findings in the study. P/LP, pathogenic or likely pathogenic; VUS, variants with unknown significance



Burrows–Wheeler Aligner (BWA, version 0.7.17-r1188) software (H. Li & Durbin, 2010). The duplicate reads were removed using Picard (version 2.21.1, <https://broadinstitute.github.io/picard/>). The pipeline of the best practice of Genome Analysis Toolkit (GATK, version 3.8) (DePristo et al., 2011) was employed to refine the alignment and call variants. The obtained SNVs and InDels were then annotated using ANNOVAR (K. Wang et al., 2010) and InterVar (version: v20180118) (Q. Li & Wang, 2017) to classify pathogenicity based on population frequency, functional impact, and so forth. For each case, we calculated the phenotype-relevant score of each identified gene with variants using Phenolyzer (Yang et al., 2015) according to the corresponding HPO words. Public databases such as OMIM (<https://www.omim.org/>), HGMD (Stenson et al., 2020), ClinVar (Landrum et al., 2016), and ClinGen (Rehm et al., 2015) were also screened for the associated disease and pathogenicity of each gene variant.

All variants were interpreted and classified according to the American College of Medical Genetics and Genomics (ACMG) guidelines (Richards et al., 2015). The filtering criteria of the detected

variants are presented in the workflow in Figure 1. The annotated variants with deleterious effects or with population frequency less than 0.01 in East Asians in gnomAD exome or genome databases were kept and then prioritized according to the phenotype correlation score. Stopgain, InDels, missense variants with CADD (Rentzsch et al., 2019) Phred score  $\geq 20$  or REVEL (Ioannidis et al., 2016) score  $> 0.5$  or MCAP (Jagadeesh et al., 2016) score  $> 0.025$ , and variants predicted splicing alternation with dbSNV ADA score and RF score  $> 0.9$  were kept in high priority. wInterVar (<https://wintervar.wglab.org/>) and varsome (version: 9.5.3, <https://varsome.com/>) were further queried to confirm the ACMG variants classification criteria of these variants.

## 2.5 | Sanger sequencing

Pathogenic or likely pathogenic SNVs and InDels were further validated using Sanger sequencing, if (1) depth  $< 20$ , or (2) minor allele fraction  $\leq 0.3$  or  $\geq 0.7$ . PCR primers were designed using the

Primer-BLAST program (Ye et al., 2012). All variants were validated by independent PCR amplification and bidirectional DNA sequencing performed on an ABI 3130 DNA analyzer (Applied Biosystems). The segregation pattern was analyzed in the pedigree to confirm the mode of inheritance.

## 2.6 | Compound heterozygous variants validation

For the patients with parents sequenced, paternal or maternal inheritances of the two heterozygous variants and whether they were in trans were confirmed. For the patients without parents' DNA samples, if the chromosomal distance between the two heterozygous variants was below 150 Bp (patient P6), the Integrative Genomics Viewer (IGV) (Thorvaldsdottir et al., 2013) was employed to view the variants-located chromosomal region of the patient's bam file to confirm in translocation; if the distance between the two heterozygous variants was below 3 Kb (patient P2 and P14), PCR primers were designed using the Primer-BLAST program (Ye et al., 2012) containing both two variants and then the PCR products were performed TA cloning (Trower & Elgar, 1994) to confirm whether the two variants were in trans.

## 2.7 | CNV analysis

For the patients without pathogenic or likely pathogenic SNVs or InDels identified, CNV analysis was further performed. The refined bam files generated in previous "Bioinformatic analysis and interpretation" step were used. CNVkit (version 0.9.6) (Talevich et al., 2016) was employed to detect germline CNVs in each patient. If the patient had family unaffected controls sequenced, the family controls were used for the reference. If only probands were sequenced without family controls, the 45 family control samples in this study were divided into male group and female group respectively to be used for the reference. The corresponding bed file associated with Agilent SureSelect Human All Exon V6 kit was used as target regions. CNVs were called using threshold parameter "-t=-1.1,-0.4,0.3,0.7" for germline variations. The called CNVs were then annotated with OMIM collected genes and ClinGen dosage-associated genes with haploinsufficiency and triplosensitivity descriptions. The CNVs were then interpreted based on the ACMG and ClinGen technical standards (Erin Rooney Riggs et al., 2019). DECIPHER database (Firth et al., 2009) was used to query any overlapped CNVs and associated phenotypes. Pathogenic or likely pathogenic CNVs were further validated via chromosomal microarray analysis (CMA) using the Infinium Asian Screening Array-24+ v1.0 (Illumina).

## 2.8 | Multidisciplinary team confirmation

Geneticists and clinicians (including PICU experts, neurologists, hematologists, etc.) at Hunan Children's Hospital cooperated to confirm

the genetic diagnosis of each identified patient. The validated variants were classified into de novo, compound heterozygous, or homozygous according to the genotype of family members. We selected the inheritance-pattern-matched and phenotype-explainable genomic variants and evaluated the genotype-phenotype relationship for diagnosis.

## 2.9 | Statistical analysis

We divided the 58 patients into two groups: 33 patients with genetic findings and 25 patients without genetic findings. According to the survival status, there were 39 deceased patients versus 19 surviving patients. Clinical features were classified according to age, affected systems, and symptoms. Statistical analyses were performed using IBM SPSS software (version 18.0; IBM Corp.). Chi-square test and Fisher's exact test were applied to compare the genetically diagnosed and undiagnosed groups and the deceased and surviving groups. The calculation of all *p* values was two-sided.

# 3 | RESULTS

## 3.1 | Clinical profile of the selected patients

Between September 20, 2014 and December 30, 2018, 5795 patients were admitted to the PICU of Hunan Children's hospital. A total of 283 have died and the rate of death is about 4.9%. Among the studied 58 patients, 39 (67.2%) died and 19 survived (Figure 1). These patients had high mortality compared to the overall patients. The median hospital turn-around time was 18.9 days (range 5–25.5), and the median turn-around time in the PICU was 13.5 days (3.8–19). Twenty-six (44.8%, 26/58) patients had severe pneumonia. Twenty-four patients had respiratory abnormalities and sepsis respectively, followed by 22 patients with metabolic disorders, 21 patients with multiple congenital anomalies (MCA), 20 patients with neuromuscular disorders, 11 patients were suspected of having immunodeficiency, nine patients with hepatic disorders, six patients with hemopathy, five patients with cardiac disorders, and four patients with renal disorders (Table S1). Severe infections, such as sepsis and pneumonia, were the most common complications associated with the found primary disorders (Table S1). Fifteen (25.9%) patients had a severe encephalic infection. Furthermore, 12 (20.7%) and 11 (19.0%) patients were suspected of having metabolic disorders or immunodeficiency combined with severe infection, respectively. The statistics of the clinical phenotypes, diagnoses, and management are shown in Table S1.

Two patients were identified pathogenic CNVs via WES and confirmed by CMA. Patient P9 had a copy loss del(7)(q36.3) with a size of 4.7 Mb and a copy gain dup(18)(q12.1q23) with a size of 48.8 Mb (Table 1). The two regions contain 40 genes and 355 genes respectively, including ClinGen haploinsufficiency gene *SHH*, *MNX1*, *SETBP1*, *SMAD4*, *TCF4*, and *ASXL3*. The detailed information of the

identified CNVs was listed in Table S2. This patient presented micrognathia, glossoptosis, hypoplastic heart, urethral stenosis, inguinal hernia, malnutrition, and global developmental delay. Patient P10 had a copy loss del(20)(q13.13) with a size of 2.9 Mb (Table 1). This region contains 57 genes including ClinGen haploinsufficiency gene *ADNP* (Table S2). He manifested hypotelorism, abnormality of eye movement, cryptorchidism, inguinal hernia, and global developmental delay. He also has lymph node tuberculosis. These identified CNVs were classified as pathogenic according to the ACMG and ClinGen criteria, and they had not been reported before.

### 3.2 | Patients received a genetic diagnosis with SNVs

Of 58 patients, 25 patients (43.1%) had pathogenic or likely pathogenic (P/LP) SNVs with relevant phenotypes and were confirmed to have genetic disorders. The features of the diagnosed patients were detailed in Table 1. The variants were from 24 genes, which primarily explained the phenotypes of 18 patients (72.0%, 18/25) and partially explain that of seven patients (Table 1; Table S1). Fourteen patients had recessively inherited variants, including 11 compound heterozygous, two homozygous, and one X-linked recessive variation (Table 2). Eleven patients had autosomal dominant inherited variants, of which five were confirmed de novo. The ACMG criteria applied for the identified variants were detailed in Table S3.

Notably, the identified genetic diseases were quite sporadic in the studied patients. Only two unrelated patients P14 and P15 carried compound heterozygous variations from the same gene *ACAT1*. P14 carried missense variants c.131T>C (p.Ile44Thr) and c.263A>C (p.Glu88Ala). P15 had a stopsense variant c.1117A>T (p.Lys373Ter) and a frameshift variant c.252del (p.Glu85LysfsTer2). Both patients presented with severe metabolic acidosis, hyponatremia, and hypokalemia (Table 1). Additional phenotypes included sepsis in P14, severe pneumonia, diarrhea, and vomiting in P15. They were surviving at the time of discharge and follow-up visit.

Patient P8 manifested mastoiditis of the middle ear, intracranial cystic lesion, hydrocephalus, subdural effusion, subarachnoid space hemorrhage, short stature, and webbed neck. She was identified two large copy losses on chromosome X expanding almost the p arm (chrX:10500-58517128) and q arm (chrX:62740577-156025374). We further analyzed her SNVs on chromosome X and found that the size of homozygous region was over 146.8 Mb, which indicated the loss of one chromosome X. Considering her phenotype of short stature and webbed neck, we thus speculated that she had Turner syndrome with a karyotype (45,X) (Table 1). As this patient was dead shortly, no blood sample was collected for karyotype analysis. In addition, she had pathogenic variant c.214C>T (p.Gln72Ter) of *TOR1A* (Table 2). This patient died of central respiratory failure with brain neoplasm.

Excepting the two novel CNVs, there was 23 novel SNVs identified among patients with a genetic diagnosis (Table 2). They were located in 15 genes. Some were located in genes with very limited

genetic variants reported previously, such as *COG4* and *ECE1*. For example, patient P21 was found compound heterozygous variants c.1255G>T (p.Glu419Ter) and c.941G>A (p.Cys314Tyr) in *COG4*. The first variant was inherited from her father, and the second was inherited from her mother. Biallelic mutations of *COG4* were revealed to cause a congenital glycosylation disorder (Type IIj) with phenotypes of varying severity, such as compound development delay, recurrent infection, hypotonia, seizures, hepatosplenomegaly, liver cirrhosis, and coagulopathy (Ng et al., 2011; Reynders et al., 2009). Patient P21 presented similar phenotypes, such as persistent jaundice, decreased liver function, abnormal coagulation, and status epilepticus. She also had disseminated intravascular coagulation and a history of recurrent bronchopneumonia and seizures. The patient finally died of liver and respiratory failure.

Some identified P/LP SNVs could not explain the patients' primary phenotypes. These SNVs were considered as secondary findings, such as SNVs from P26 and P27. The two patients both had a severe infection. Patient P26 had c.296G>A (p.Arg99His) in gene *SCN9A* with uncertain significance. He also had a likely pathogenic variant c.890G>T (p.Cys297Phe) in gene *IDH1* which has susceptibility to somatic glioma. The patient presented acute intracranial hypertension, fever, bilateral convulsive seizures, loss of consciousness. Computerized tomography (CT) revealed strips of dense shadows under the cortex of the frontal lobe on both sides and the globus pallidus on both sides. The large wing of the right sphenoid bone and the outer wall of the orbit could see a round-shaped bone destruction area with a clear boundary, about 1.2 × 1.2 × 1.4 cm (CT value is about 26–35HU). Patient P27 had a likely pathogenic variant c.1114C>T (p.Leu342Phe) in gene *G6PD* (Table 2). This variant was reported relating to glucose phosphate dehydrogenase deficiency (Vulliamy et al., 1993). This patient was a boy and was confirmed to have *G6PD* deficiency. He presented sepsis, diarrhea, fever, skin rash, hypersensitivity drug reaction, anemia, and malnutrition. No hemolytic anemia was found during his hospitalization period. This variant was inherited from his mother and was considered a second finding.

### 3.3 | Six patients with VUS variants

In addition to P/LP variants, six patients (P28-P33) had VUS variants (Table 1, Table 2). P28 had one VUS in gene *PLCG2* associated with autosomal dominant autoinflammation disease (MIM# 614878), and the patient presented interstitial pneumonitis, impaired B cell memory cells, and recurrent infection. P29 had one VUS in gene *PHOX2B* related to autosomal dominant central hypoventilation syndrome (MIM# 209880) and he presented idiopathic respiratory distress syndrome, alveolar hypoventilation, and shallow breathing. The patient also had one VUS in *DSP* related to autosomal dominant arrhythmogenic right ventricular dysplasia 8 (MIM# 607450), dilated cardiomyopathy (MIM# 615821), and keratosis palmoplantaris striata II (MIM# 621908), and he presented congenital heart defects. P30 had one VUS in gene *PARN* related to autosomal dominant pulmonary fibrosis and/or bone marrow failure (MIM# 616371). She manifested

TABLE 1 Characteristics of 33 patients with genetic findings

Category	Patient	Sex	Age	Hospital turn-around time (days)	PICU Turn-around time (days)	Gene	Gene matched phenotypes	Additional features	Phenotype explained	Severe infection?	Prognosis	Possible clinical interventions
RES	P1	F	3m	44	44	CFTR	Fibrocystic lung disease: chronic bronchopulmonary infection, pseudomonas colonization		Primary	Pneumonia	Deceased	Protective quarantine to avoid severe infection
	P2	M	2m	9	9	MARS1, DNAH11 (VUS)	Interstitial lung and liver disease: hepatomegaly, liver dysfunction, anemia, respiratory insufficiency, dyspnea, cough	Inguinal hernia, hydrocele, testis	Primary	Pneumonia	Deceased	
	P3	F	6m	18	6	RTEL1	Pulmonary fibrosis, IRDS,	Leukocytosis	Primary	Sepsis, pneumonia	Deceased	
	P4	M	15y	20	20	AQP5	Palmoplantar keratoderma, Bothnian type	Seizures, hemophagocytosis	Partial	Sepsis, pneumonia, encephalitis	Deceased	
NEU	P5	F	4m	7	7	PRRT2	Seizures	Diarrhea	Primary		Surviving	Antiepileptic
	P6	M	3m	40	40	NFU1	Multiple mitochondrial dysfunctions syndrome: pulmonary arterial hypertension, respiratory failure, lethargy, decreasing responsiveness, lactic acidosis, Infantile encephalopathy		Primary		Deceased	
	P7	M	9m	1	1	TBCK	Seizures, periventricular white matter abnormalities		Partial	Sepsis	Deceased	
MCA	P8 <sup>a</sup>	F	9y11m	29	29	del(X), TOR1A	Turner syndrome: short stature, webbed neck, hypotonia, recurrent otitis media, cystic hygroma	Brain neoplasm	Primary		Deceased	



TABLE 1 (Continued)

Category	Patient	Sex	Age	Hospital turn-around time (days)	PICU Turn-around time (days)	Gene	Gene matched phenotypes	Additional features	Phenotype explained	Severe infection?	Prognosis	Possible clinical interventions
	P9 <sup>b</sup>	M	9m	17	4	CNV: del(7)(q36.3), ins(18)(q12.1-q23)	Congenital abnormalities, global developmental delay		Primary	Pneumonia	Surviving	
	P10	M	3m	25	19	CNV: del(20)(q13.13)	Congenital abnormalities, global developmental delay	Lymph node tuberculoses	Primary	Sepsis, pneumonia	Deceased	
	P11	M	6m		18	ECE1	Hirschsprung disease, cardiac defects, and autonomic dysfunction: ventricular septal defect, atrial septal defect, failure to thrive in infancy, vomiting, sepsis	IRDS	Primary	Sepsis, pneumonia	Deceased	
	P12	F	1y2m	23	5	ARX	Agenesis of the corpus callosum	Gastroenteritis, acute kidney injury	Partial	Sepsis	Surviving	
	P13	M	1m	7	7	ZEB2	Mowat-Wilson syndrome: aganglionic megacolon, abnormal facial shape, ventricular septal defect, patent ductus arteriosus, megacolon		Primary	Pneumonia, enterocolitis	Deceased	
MET	P14	F	4m	98	98	ACAT1	Beta-ketothiolase deficiency: ketoacidosis, infections may precipitate ketotic episodes		Primary	Sepsis	Surviving	Limited protein intake and low leucine dietary
	P15	M	1y4m	2	2	ACAT1	Beta-ketothiolase deficiency: metabolic acidosis, vomiting, intellectual disability, ketoacidosis		Primary	Pneumonia	Surviving	Limited protein intake and low leucine dietary
	P16	M	10m	22	8	CPS1	Carbamoyl-Phosphate Synthase I Deficiency: coma, cerebral edema, respiratory alkalosis, hyperammonemia		Primary	Encephalitis	Deceased	Early intervention when hyperammonemia

(Continues)

TABLE 1 (Continued)

Category	Patient	Sex	Age	Hospital turn-around time (days)	PICU Turn-around time (days)	Gene	Gene matched phenotypes	Additional features	Phenotype explained	Severe infection?	Prognosis	Possible clinical interventions
	P17	F	1y3m	35	35	AGL	Glycogen Storage Disease: hepatomegaly, hepatic fibrosis, hypoglycemia, elevated transaminases, increased serum creatine kinase	Enteritis	Primary		Surviving	Dietary
HEM	P18	F	1y2m	1	1	VWF	Von Willebrand Disease Type 3: prolonged bleeding time, prolonged bleeding after surgery or trauma, intracranial hemorrhage		Primary		Deceased	VMF VIII supply at regular period
	P19	F	1y11m	22	10	KRAS	Acute myelogenous leukemia		Primary	Sepsis, pneumonia	Deceased	
HEP	P20	F	3y7m	4	4	NBAS	Infantile liver failure syndrome: vomiting, jaundice, hypoglycemia, lethargy, hyperammonemia, coagulopathy, and elevated liver enzymes during episodes, recurrent episodes of liver failure during intercurrent infections, complete recovery during intervals		Primary		Deceased	Liver transplantation
	P21	F	9m	1	1	COG4	Glycosylation IIj: hepatomegaly, liver failure, delayed psychomotor development, seizures, decreased coagulation factors, abnormal liver enzymes, microcephaly		Primary		Deceased	



**TABLE 1** (Continued)

Category	Patient	Sex	Age	Hospital turn-around time (days)	PICU Turn-around time (days)	Gene	Gene matched phenotypes	Additional features	Phenotype explained	Severe infection?	Prognosis	Possible clinical interventions
REN	P22	F	1y2m	12	12	INVS	Kidney consumption, infantile nephronophthisis, senior-Loken syndrome: hypertension, respiratory failure, renal failure, heart failure, elevated serum creatinine, situs inversus and valvular or ventricular septal defects, global developmental delay	Pulmonary edema	Primary	Sepsis	Deceased	
CAR	P23	F	2m	6	6	TNNI2	Cardiomyopathy: congestive heart failure, left ventricular dilation		Primary		Deceased	
IMM	P24	M	6m	53	46	LIG4	Immunodeficiency: recurrent severe infection, tuberculous lymphadenitis	Polydactyly	Primary	Sepsis, pneumonia	Deceased	Bone marrow transplantation
	P25	M	5m	5	5	NLRP12	Familial Cold Autoinflammatory Syndrome: splenomegaly, rash, fever	Hypoplastic heart, hydrocele testis, laryngomalacia, tracheomalacia, hyperpigmented nevi, Mongolian blue spot, global developmental delay	Partial	Pneumonia	Surviving	
Other secondary findings	P26	M	5y8m	11	11	IDH1, SCN9A	Paroxysmal extreme pain disorder (Generalized epilepsy with febrile seizures plus, type 7); susceptibility to somatic glioma: seizures, febrile seizures, acute intracranial hypertension with abnormal compact image and bone destruction in CT		Partial	Encephalitis	Deceased	

(Continues)

TABLE 1 (Continued)

Category	Patient	Sex	Age	Hospital turn-around time (days)	PICU Turn-around time (days)	Gene	Gene matched phenotypes	Additional features	Phenotype explained	Severe infection?	Prognosis	Possible clinical interventions
	P27	M	8m	12	12	G6PD	Glucose-6-phosphate dehydrogenase (G6PD) deficiency	Protracted diarrhea, hypersensitivity drug reaction	Partial	Sepsis	Surviving	
Other VUUs	P28	F	7y10m	9	9	PLCG2	Autoinflammation, antibody deficiency, and immune dysregulation syndrome: interstitial pneumonitis, impaired B cell memory cells, recurrent infection		Primary	Sepsis	Deceased	
	P29	M	6m	46	28	PHOX2B, DSP	IRDS; congenital heart defects: alveolar hypoventilation, shallow breathing		Primary	Sepsis, pneumonia	Deceased	
	P30	F	11y8m	1	1	PARN	Interstitial pneumonitis: pulmonary fibrosis, immunodeficiency, anemia		Partial	Pneumonia	Deceased	
	P31	M	1m	3	3	MYH6	Atrial septal defect, dilated cardiomyopathy, hypertrophic cardiomyopathy: ventricular septal defect, patent foramen ovale		Primary	Pneumonia	Surviving	Respiratory support
	P32	M	1m	24	24	VCP, SCN5A	Amyotrophy, inclusion body myopathy with early-onset Paget disease and frontotemporal dementia 1: muscle weakness, dysphagia, dyspnea, motor neuron dysfunction	Laryngomalacia, cartilaginous trachea	Primary	Pneumonia	Deceased	

TABLE 1 (Continued)

Category	Patient	Sex	Age	Hospital turn-around time (days)	PICU Turn-around time (days)	Gene	Gene matched phenotypes	Additional features	Phenotype explained	Severe infection?	Prognosis	Possible clinical interventions
P33	M	2y-	8m	7	7	ABCC2	Dubin-Johnson syndrome; cholestasis, jaundice, conjugated hyperbilirubinemia		Primary	Pneumonia	Deceased	

Abbreviations: CAR, cardiac disorder; HEM, hemopathy; HEP, hepatic disorder; IMM, immunodeficiency; IRDS, infantile respiratory distress syndrome; m, month for age; MET, metabolic disorder; NEU, neuromuscular disorder; REN, renal disorder; RES, respiratory disorder; VUS, variants uncertain significance; y, year.

<sup>a</sup>This patient died of acute tricaral hypertension and respiratory failure. She was further confirmed Turer syndrome after died via WES by CNV analysis and homozygous SNVs analysis. Her associated phenotypes including short stature, webbed neck, etc.

<sup>b</sup>This patient was identified an abnormal karyotype 7q+ firstly when admitted to our hospital, and was further detected CNV of del(7)(q36.3) and ins(18)(q12.1q23).

pulmonary fibrosis, immunodeficiency, and anemia. P31 had one VUS in gene *MYH6* related to the atrial septal defect (MIM# 614089), autosomal dominant dilated cardiomyopathy (MIM# 613252), and hypertrophic cardiomyopathy (MIM# 613251). The patient presented ventricular septal defect and patent foramen ovale. P32 had one VUS in gene *VCP* related to autosomal dominant inclusion body myopathy with early-onset Paget disease and frontotemporal dementia (MIM# 167320). The patient presented muscle weakness, dysphagia, dyspnea, and motor neuron dysfunction. He also had one VUS in *SCN5A*. P33 had compound heterozygous variants in gene *ABCC2*, and one is likely pathogenic and the other is VUS. *ABCC2* is related to autosomal recessive Dubin-Johnson syndrome. The patient manifested cholestasis, jaundice, and conjugated hyperbilirubinemia. These VUS-associated genes can primarily explain the phenotypes of five patients (P28, P29, P31–P33). As the lack of the DNA samples of their parents, whether these variants were de novo could not be determined. Their pathogenicity needs to be further investigated.

### 3.4 | Phenotype characteristics of patients with genetic findings

Of the 33 patients with genetic findings, 24 (72.7%) patients died, and nine (27.3%) survived (Figure 1). Of the 27 patients who received a genetic diagnosis with P/LP variants, the genotypes explained the primary clinical phenotypes of 26 patients (78.8%, 26/33). In which, 13 (50%, 13/26) patients were complicated by severe infection and 10 were deceased. Only P14 and P15 with *ACAT1* gene variants and P8 with CNV were surviving. Six patients (P2, P3, P10, P11, P22, P24) had phenotypes apart from the related phenotypes of the identified genetic diseases (Table 1). For example, P2 had an inguinal hernia and hydrocele testis, in addition to interstitial lung and liver disease caused by compound heterozygous mutation in the *MARS1* gene.

In the six patients (P4, P7, P12, P25, P26, P27) who received a genetic diagnosis, their phenotypes could be partially explained by the identified genotypes. They all had severe infections. P4, P12, P25, and P27 had additional features apart from the identified genetic diseases. P4, P7, and P26 were deceased finally. In the six patients with VUS findings, only P31 was surviving with *MYH6* variant accompanied pneumonia. Others died complicated by severe infection.

Overall, near half of the 27 patients with a genetic diagnosis had respiratory defects (44.4%) or severe pneumonia (48.1%) (Table S1). Nineteen patients (70.4%) had severe infections and 13 of them died (68.4%) (Table S4). Ten patients have phenotypes unexplained by the identified genotypes. Nineteen patients died finally.

## 4 | DISCUSSION

Genetic diseases, particularly rare diseases, usually have early onset. It was estimated that 50%–75% of rare diseases affect children (Wright et al., 2018). Many of them impair multisystem and have a range of severe phenotypes. Rare diseases become an important

TABLE 2 Information of the detected variants of the patients with genetic findings

Patient ID	ES family	Inheritance/ Zygoty	Gene name	HGVS transcript coding change	HGVS predicted protein change	Allele origin	Variant classification <sup>a</sup>	Novel or reported
P1	proband	het	CFTR	NM_000492.3:c.3196C>T and c.595C>T	p.(Arg1066Cys) and p.(His199Tyr)	Unknown	LP	HGMD and ClinVar recorded
P2	proband	compHet	MARS1	NM_004990.3:c.1547A>G and c.1793G>A	p.(Tyr516Cys) and p.(Arg598His)	Unknown	LP	Novel, p.(Arg598Cys) reported in PMID:32833345.
P2	proband	compHet?	DNAH11	NM_001277115.1:c.6934T>C and c.11352C>A	p.(Ser2312Pro) and p.(Phe3784Leu)	Unknown	VUS	Novel
P3	proband	het	RTEL1	NM_032957.4:c.709C>T	p.(Arg237Trp)	Unknown	LP	HGMD damaging, PMID:26022962
P4	proband	het	AQP5	NM_001651.3:c.526G>A	p.(Gly176Arg)	Unknown	LP	Novel
P5	proband	het	PRRT2	NM_145239.2:c.971dup	p.(Val325SerfsTer16)	Unknown	P	Novel
P6	proband	compHet	NFU1	NM_015700.3:c.473+5G>A and c.473G>A	p.? and p.(Arg158Gln)	Unknown	P	HGMD and ClinVar damaging
P7	trio + 2 siblings	hom	TBCK	NM_001163436.2:c.389T>A	p.(Ile130Asn)	Paternal + maternal	LP	Novel
P8	proband	het	TOR1A	NM_000113.2:c.214C>T	p.(Gln72Ter)	Unknown	P	Novel
P8	proband	het	APOE	NM_000041.3:c.784G>A and c.787G>A	p.(Glu262Lys) and p.(Glu263Lys)	Unknown	VUS	ClinVar and HGMD pathogenic
P11	proband + mother	het	ECE1	NM_001397.2:c.1966G>A	p.(Gly656Arg)	Unknown	LP	Novel
P12	trio	de novo	ARX	NM_139058.2:c.947del	p.(Gly316AlafsTer9)	de novo	P	Novel
P13	trio	de novo	ZEB2	NM_014795.3:c.2717del	p.(Pro906LeufsTer24)	de novo	P	Novel
P14	proband	compHet	ACAT1	NM_000019.3:c.131T>C and c.263A>C	p.(Ile44Thr) and p.(Glu88Ala)	Unknown	LP	Novel
P15	trio	compHet	ACAT1	NM_000019.3:c.1117A>T and c.252del	p.(Lys373Ter) and p.(Glu85LysfsTer2)	Paternal + maternal	P	Novel
P16	trio	compHet	GPS1	NM_001875.4:c.1145C>T and c.1164+2T>C	p.(Pro382Leu) and p.?	Paternal + maternal	LP, P	Novel
P17	trio	compHet	AGL	NM_000646.2:c.1687+1G>T and c.2633+5G>C	p.?	Maternal + paternal	P, LP	HGMD and ClinVar damaging, novel
P18	trio	compHet	VWF	NM_000552.4:c.2635G>A and c.6963del	p.(Asp879Asn) and p.(Glu2322SerfsTer9)	Maternal + paternal	P, P	Reported, Novel

TABLE 2 (Continued)

Patient ID	ES family	Inheritance/ Zygoty	Gene name	HGVS transcript coding change	HGVS predicted protein change	Allele origin	Variant classification <sup>a</sup>	Novel or reported
P19	trio	de novo	KRAS	NM_004985.4:c.35G>A	p.(Gly12Asp)	de novo	P	ClinVar and HGMD
P20	trio	compHet	NBAS	NM_015909.3:c.2630G>T and c.4288C>T	p.(Gly877Val) and p.(Gln1430Ter)	Maternal + paternal	LP, P	Novel
P21	trio	compHet	COG4	NM_015386.2:c.1255G>T and c.941G>A	p.(Glu419Ter) and p.(Cys314Tyr)	Paternal + maternal	P, LP	Novel
P22	proband	hom	INVS	NM_014425.3:c.2887C>T	p.(Gln963Ter)	biparental?	P	Novel
P23	trio	de novo	TNNT2	NM_000364.3:c.650_652del	p.(Lys217del)	de novo	P	Reported
P24	trio	compHet	LIG4	NM_002312.3:c.833G>T and c.1144_1145del	p.(Arg278Leu) and p.(Leu382GlufsTer5)	Paternal + maternal	P, P	HGMD damaging, ClinVar
P25	proband	het	NLRP12	NM_144687.3:c.1742G>A	p.(Trp581Ter)	Unknown	P	Reported
P26	proband	het	IDH1	NM_005896.3:c.890G>T	p.(Cys297Phe)	Unknown	LP	Novel
P26	proband	het	SCN9A	NM_002977.3:c.296G>A	p.(Arg99His)	Unknown	VUS	ClinVar VUS
P27	trio	XLR	G6PD	NM_000402.4:c.1114C>T	p.(Leu372Phe)	Maternal	LP	HGMD damaging
P28	proband	het	PLCG2	NM_002661.4:c.64C>A	p.(Leu22Met)	Unknown	VUS	Novel
P29	proband	het	PHOX2B	NM_003924.3:c.829C>A	p.(Pro277Thr)	Unknown	VUS	Novel
P29	proband	het	DSP	NM_004415.3:c.38C>T	p.(Thr13Ile)	Unknown	VUS	HGMD damaging
P30	proband	het	PARN	NM_002582.3:c.1385A>G	p.(Tyr462Cys)	Unknown	VUS	Novel
P31	proband	het	MYH6	NM_002471.3:c.4496C>A	p.(Thr1499Asn)	Unknown	VUS	Novel
P32	proband	het	SCN5A	NM_000335.4:c.3067C>T	p.(Arg1023Cys)	Unknown	VUS	HGMD damaging
P32	proband	het	VCP	NM_007126.4:c.697A>G	p.(Ile233Val)	Unknown	VUS	ClinVar VUS
P33	proband	compHet?	ABCC2	NM_000392.5:c.4239_4240dup and c.2755T>A	p.(His1414LeufsTer18) and p.(Ser919Thr)	Unknown	P, VUS	ClinVar pathogenic, ClinVar uncertain
P9	proband		CNV				P	Novel
P10	trio	de novo	CNV				P	Novel

Note: compHet: compound heterozygotes; compHet?: supposed to be compound heterozygous variants but have not been confirmed in trans. P15–P18, P20, P21, and P24 were confirmed in trans by parental sequences (trio). P2 and P14 were confirmed in trans by TA cloning (distance < 3 Kb). P6 was confirmed in trans by IGV (distance < 150 bp).

<sup>a</sup>Please see Table S3 for the matched details of ACMG variant classification criteria.

reason for pediatric hospital admission, and they account for 20%–35% of the early death in children less than 1 year old (Dodge et al., 2011; Kingsmore et al., 2020; Yang et al., 2020). In PICUs, a considerable proportion of patients may have a genetic etiology but have not undergone gene testing yet, particularly those who died without a clear etiology in some developing areas. This retrospective study employed WES integrating comprehensive SNV and CNV analyses, and revealed that 27 of the 58 regional PICU patients had genetic diseases. The identified disease-causing genes were quite sporadic. Our findings expanded the genotypes and phenotypes of at least 19 rare diseases, including 23 novel SNVs from 15 genes and two novel CNVs. 70.4% of the patients with genetic disorders deceased. Severe pneumonia or sepsis was a common accompanied symptom along with a genetic condition that resulted in high mortality.

This regional PICU represents a subgroup of patients who are born with a genetic condition and live in developing areas. In this study, the investigated patients were all transferred from local prefectural hospitals with specific disease progression. The median hospital turn-around time was long as 18.9 days. Some had complex clinical manifestations, and some phenotypes might be atypical. Of the investigated patients, a majority had multisystem abnormalities involving respiratory, neuromuscular, MCA, metabolic, hepatic, renal, cardiac, or hematological systems. WES and WGS provide a powerful early diagnosis method for patients with rare diseases with uncertain etiology, atypical clinical manifestations, or complex expression primarily (Johansen Taber et al., 2014; Kingsmore & Saunders, 2011; Pinxten & Howard, 2014; E. D. Smith et al., 2019). In our study, 56.9% of the participated patients had genetic findings and 46.6% can make a genetic diagnosis. The detected genetic diseases were various and only two patients were caused by the same gene.

In addition to atypical phenotypes and sporadically spread genetic disorders, severe infection (including sepsis, severe pneumonia, or encephalic infection) was found in the majority of our patients. These made the clinical manifestations more complex and critical. In a review by Hotchkiss et al. (2016), the mortality rates of sepsis and septic shock in the general population were 15%–25% and 30%–50%, respectively. Another review summarized that the hospital mortality of severe sepsis was 10.3% in US children (Watson & Carcillo, 2005). Among 123 patients with sepsis in the same PICU department in our hospital in 2019, the mortality rates of sepsis, severe sepsis, and septic shock were 5.5%, 25%, and 27.3%, respectively (unpublished data). The mortality rate of severe pneumonia was approximately 4.2% in 2015 in developing countries (McAllister et al., 2019). This study found high mortality (69%) in patients with severe infection, even excluding immunodeficient patients. In the patients who had severe infections with underlying genetic diseases primarily explained their phenotypes, the mortality increased to 76.9%. Previously, Watson and Carcillo (2005) summarized that the hospital mortality of US children with severe sepsis was 7.8% in previously healthy children, and increased to 12.8% in children with underlying disease. This probably indicates that severe infection concurrently contributes to the death of patients with genetic etiology.

An early precise diagnosis could improve the surviving rate and reduce the admission rate in the PICU. A recent study revealed that the mortality in infants with genetic diseases was 21.6% (205 of 948 infant deaths) (Kingsmore et al., 2020). Jacob et al. (2015) found that only 5% of 641 deceased NICU patients had genetic defects. In a retrospective study of 278 infants from the NICU, PICU, and cardiac ICU by WES, 48.1% of 81 deceased patients were diagnosed with genetic conditions (Meng et al., 2017). Our study revealed that 68.4% of deceased patients had genetic conditions, which is greater than the results of previous studies. This is probably due to the bias in selecting participants (67.2% deaths vs. 4.9% overall deaths during the same period). Respiratory system disorders, neuromuscular, and sepsis were more common in the deceased patients than in the surviving patients. Similarly, research on mortality in approximately 467,000 patients in a pediatric emergency department revealed that the main causes of death were respiratory, neuromuscular, or cardiac disorders and sepsis (Zhu et al., 2015). An earlier differential diagnosis and timely management could save lives or improve living quality.

Overall, the expanded genotypes and phenotypes in this study would facilitate precise diagnosis and subsequent treatments in clinical practice. For some diseases, early diagnosis can allow for interventions that reduce unnecessary testing and treatment. For some families, a clear diagnosis and poor prognosis are closely related to the parent's decision to pursue aggressive or palliative care. In our study, at least 11 children could have undergone appropriate clinical interventions according to the genetic diagnosis. Six lives could have been saved, and the quality of life of five children could have been improved. Furthermore, with known genetic variants and carrier status determined by genomic testing, it would be beneficial for the related families to undergo prenatal diagnosis and screening to avoid giving birth to babies with genetic defects. Thus, morbidity and mortality in newborns would be reduced, as well as the burden of disease.

Since this was a retrospective study, no instance data of clinical management could be obtained after receiving a molecular diagnosis. Other limitations include the following: first, complete data were not available from some patients to further evaluate the pathogenicity of the detected variants. A large number of detected variants were classified as having uncertain significance. For example, some cases lacked the samples of parents. Some lacked the related clinical data to confirm the genotype-phenotype correlations, including physical examinations, enzyme assays, imaging studies, or pathological detections. Some families of the patients were difficult to contact to obtain detailed follow-up information on disease progress and individual development. Second, WES-based technology cannot detect genomic variants in noncoding regions or structural variants, such as translocation, inversion, and mosaic variants. WGS could improve the diagnosis rate. For some undiagnosed neuromuscular cases, a special mitochondrial test would be a good choice. Third, the phenotypes of some patients might not be examined thoroughly due to limited time or facilities. Some phenotypes might be incomplete or late-onset due to individual differences. Forth, due to the limited sample number,

there were a few patients with certain clinical criteria (such as hematopoiesis or renal diseases), and thus some statistical data might not be very strong. We believe further examinations and analyses could enhance genetic diagnosis more comprehensively and provide more sophisticated guidance for clinical practice.

## 5 | CONCLUSION

WES integrated with CNV analysis helped to clarify the etiology of dozens of the regional PICU patients. Our findings expanded the genotypic and phenotypic spectrum over 19 rare diseases and will provide a basis for clinical practice in earlier preventing, diagnosis, and management of such rare diseases.

## ACKNOWLEDGMENTS

This study was supported by the Key Laboratory of Emergency Medicine for Children (grant no.: 2018TP1028), and the Ministry of Science and Technology in China (grant no.:2012BAI04B02). The authors would like to thank the Pediatrics Research Institute of Hunan Province for its contribution of valuable assistance with the laboratory and field tests. They also would like to express our sincere gratitude to all those who helped during the writing of this thesis.

## CONFLICT OF INTERESTS

The authors declare that there are no conflict of interests.

## AUTHOR CONTRIBUTIONS

Conceptualization and investigation: Juan Liu, Yu Zheng, Yongjia Yang, Zhenghui Xiao, Yimin Zhu, and Xiulan Lu. Funding acquisition, investigation, supervision, resources acquisition, methodology, and validation: Zhenghui Xiao, Yimin Zhu, and Xiulan Lu. Original draft writing and editing, data analysis, and visualization: Yu Zheng and Juan Liu. Resources collection and data validation: Xiulan Lu, Yongjia Yang, Zhenqing Luo, and Yu Peng. Manuscript review and editing: Xiulan Lu, Zhenghui Xiao, Yimin Zhu, and Yongjia Yang. Jiaotian Huang, Desheng Zhu, and Ping Zang contributed to ideas refining, additional analyses, and finalization. All authors read and approved the final manuscript.

## DATA AVAILABILITY STATEMENT

The novel variants found in this study have been submitted to ClinVar (Submission ID: SUB9827004, ClinVar accession: from SCV001737514 to SCV001737542). Other datasets used or analyzed during the current study are available from the corresponding author on reasonable request. Some or all data, models, or codes generated or used during the study are available from the corresponding author by request.

## ETHICS STATEMENT

The samples were obtained appropriate informed consent from all participants.

## ORCID

Yu Zheng  <https://orcid.org/0000-0002-2511-961X>

Yongjia Yang  <http://orcid.org/0000-0002-8673-1589>

Xiulan Lu  <http://orcid.org/0000-0002-7297-4360>

## REFERENCES

- Arts, P., Simons, A., AlZahrani, M. S., Yilmaz, E., Alldrissi, E., van Aerde, K. J., Alenezi, N., AlGhamdi, H. A., AlJubab, H. A., Al-Hussaini, A. A., AlManjomi, F., Alsaad, A. B., Alsalem, B., Andijani, A. A., Asery, A., Ballourah, W., Bleeker-Rovers, C. P., van Deuren, M., van der Flier, M., ... Hoischen, A. (2019). Exome sequencing in routine diagnostics: A generic test for 254 patients with primary immunodeficiencies. *Genome Medicine*, 11(1), 38. <https://doi.org/10.1186/s13073-019-0649-3>
- Australian Genomics Health Alliance Acute Care Flagship, Lunke, S., Eggers, S., Wilson, M., Patel, C., Barnett, C. P., Pinner, J., Sandaradura, S. A., Buckley, M. F., Krzesinski, E. I., de Silva, M. G., Brett, G. R., Boggs, K., Mowat, D., Kirk, E. P., Adès, L. C., Akesson, L. S., Amor, D. J., Ayres, S., ... Stark, Z. (2020). Feasibility of ultra-rapid exome sequencing in critically ill infants and children with suspected monogenic conditions in the Australian Public Health Care System. *Journal of the American Medical Association*, 323(24), 2503–2511. <https://doi.org/10.1001/jama.2020.7671>
- Borghesi, A., Mencarelli, M. A., Memo, L., Ferrero, G. B., Bartuli, A., Genuardi, M., Villani, A., Renieri, A., & Corsello, G., their respective Scientific Societies. (2017). Intersociety policy statement on the use of whole-exome sequencing in the critically ill newborn infant. *Italian Journal of Pediatrics*, 43(1), 100. <https://doi.org/10.1186/s13052-017-0418-0>
- Clark, M. M., Hildreth, A., Batalov, S., Ding, Y., Chowdhury, S., Watkins, K., Ellsworth, K., Camp, B., Kint, C. I., Yacoubian, C., Farnaes, L., Bainbridge, M. N., Beebe, C., Braun, J., Bray, M., Carroll, J., Cakici, J. A., Caylor, S. A., Clarke, C., ... Kingsmore, S. F. (2019). Diagnosis of genetic diseases in seriously ill children by rapid whole-genome sequencing and automated phenotyping and interpretation. *Science Translational Medicine*, 11(489). <https://doi.org/10.1126/scitranslmed.aat6177>
- ClinGen. <https://clinicalgenome.org/>
- ClinVar. <https://www.ncbi.nlm.nih.gov/clinvar/>
- Daoud, H., Luco, S. M., Li, R., Bareke, E., Beaulieu, C., Jarinova, O., Carson, N., Nikkel, S. M., Graham, G. E., Richer, J., Armour, C., Bulman, D. E., Chakraborty, P., Geraghty, M., Lines, M. A., Lacaze-Masmonteil, T., Majewski, J., Boycott, K. M., & Dymont, D. A. (2016). Next-generation sequencing for diagnosis of rare diseases in the neonatal intensive care unit. *Canadian Medical Association Journal / Journal de l'Association Medicale Canadienne*, 188(11), E254–e260. <https://doi.org/10.1503/cmaj.150823>
- DECIPHER, <https://www.deciphergenomics.org/>
- DePristo, M. A., Banks, E., Poplin, R., Garimella, K. V., Maguire, J. R., Hartl, C., Philippakis, A. A., del Angel, G., Rivas, M. A., Hanna, M., McKenna, A., Fennell, T. J., Kernytsky, A. M., Sivachenko, A. Y., Cibulskis, K., Gabriel, S. B., Altshuler, D., & Daly, M. J. (2011). A framework for variation discovery and genotyping using next-generation DNA sequencing data. *Nature Genetics*, 43(5), 491–498. <https://doi.org/10.1038/ng.806>
- Dodge, J. A., Chigladze, T., Donadieu, J., Grossman, Z., Ramos, F., Serlicorni, A., Siderius, L., Stefanidis, C. J., Tasic, V., Valiulis, A., & Wierzbica, J. (2011). The importance of rare diseases: From the gene to society. *Archives of Disease in Childhood*, 96(9), 791–792. <https://doi.org/10.1136/adc.2010.193664>
- Elliott, A. M., du Souich, C., Lehman, A., Guella, I., Evans, D. M., Candido, T., Tooman, L., Armstrong, L., Clarke, L., Gibson, W., Gill, H., Lavoie, P. M., Lewis, S., McKinnon, M. L., Nikkel, S. M., Patel, M.,



- Solimano, A., Synnes, A., Ting, J., ... Osiovich, H. (2019). RAPIDOMICS: Rapid genome-wide sequencing in a neonatal intensive care unit—successes and challenges. *European Journal of Pediatrics*, 178(8), 1207–1218. <https://doi.org/10.1007/s00431-019-03399-4>
- Erin Rooney Riggs, M., Andersen, E. F., Cherry, A. M., Kantarci, S., Kearney, H., Patel, A., Raca, G., Ritter, D. I., South, S. T., Thorland, E. C., Pineda-Alvarez, D., Aradhya, S., & Martin, C. L. (2019). Technical standards for the interpretation and reporting of constitutional copy-number variants: A joint consensus recommendation of the American College of Medical Genetics and Genomics (ACMG) and the Clinical Genome Resource (ClinGen). *Genetics in Medicine*. <https://www.nature.com/articles/s41436-019-0686-8>
- Farnaes, L., Hildreth, A., Sweeney, N. M., Clark, M. M., Chowdhury, S., Nahas, S., Cakici, J. A., Benson, W., Kaplan, R. H., Kronick, R., Bainbridge, M. N., Friedman, J., Gold, J. J., Ding, Y., Veeraraghavan, N., Dimmock, D., & Kingsmore, S. F. (2018). Rapid whole-genome sequencing decreases infant morbidity and cost of hospitalization. *NPJ Genomic Medicine*, 3, 10. <https://doi.org/10.1038/s41525-018-0049-4>
- Firth, H. V., Richards, S. M., Bevan, A. P., Clayton, S., Corpas, M., Rajan, D., Van Vooren, S., Moreau, Y., Pettett, R. M., & Carter, N. P. (2009). DECIPHER: Database of chromosomal imbalance and phenotype in humans using Ensembl resources. *American Journal of Human Genetics*, 84(84), 524–533.
- French, C. E., Delon, I., Dolling, H., Sanchis-Juan, A., Shamardina, O., Mégy, K., Abbs, S., Austin, T., Bowdin, S., Branco, R. G., Firth, H., NIHR BioResource—Rare Disease, Next Generation Children Project, Rowitch, D. H., & Raymond, F. L. (2019). Whole genome sequencing reveals that genetic conditions are frequent in intensively ill children. *Intensive Care Medicine*, 45(5), 627–636. <https://doi.org/10.1007/s00134-019-05552-x>
- GnomAD. <https://gnomad.broadinstitute.org/>
- Gubbels, C. S., VanNoy, G. E., Madden, J. A., Copenheaver, D., Yang, S., Wojcik, M. H., Gold, N. B., Genetti, C. A., Stoler, J., Parad, R. B., Roumiantsev, S., Bodamer, O., Beggs, A. H., Juusola, J., Agrawal, P. B., & Yu, T. W. (2020). Prospective, phenotype-driven selection of critically ill neonates for rapid exome sequencing is associated with high diagnostic yield. *Genetics in Medicine*, 22(4), 736–744. <https://doi.org/10.1038/s41436-019-0708-6>
- HGMD. <http://www.hgmd.cf.ac.uk/ac/validate.php>
- Hotchkiss, R. S., Moldawer, L. L., Opal, S. M., Reinhart, K., Turnbull, I. R., & Vincent, J. L. (2016). Sepsis and septic shock. *Nature Reviews Disease Primers*, 2, 16045.
- Ioannidis, N. M., Rothstein, J. H., Pejaver, V., Middha, S., McDonnell, S. K., Baheti, S., Musolf, A., Li, Q., Holzinger, E., Karyadi, D., Cannon-Albright, L. A., Teerlink, C. C., Stanford, J. L., Isaacs, W. B., Xu, J., Cooney, K. A., Lange, E. M., Schleutker, J., Carpten, J. D., ... Sieh, W. (2016). REVEL: An ensemble method for predicting the pathogenicity of rare missense variants. *American Journal of Human Genetics*, 99(4), 877–885. <https://doi.org/10.1016/j.ajhg.2016.08.016>
- Jacob, J., Kamitsuka, M., Clark, R. H., Kelleher, A. S., & Spitzer, A. R. (2015). Etiologies of NICU deaths. *Pediatrics*, 135(1), e59–e65. <https://doi.org/10.1542/peds.2014-2967>
- Jagadeesh, K. A., Wenger, A. M., Berger, M. J., Guturu, H., Stenson, P. D., Cooper, D. N., Bernstein, J. A., & Bejerano, G. (2016). M-CAP eliminates a majority of variants of uncertain significance in clinical exomes at high sensitivity. *Nature Genetics*, 48(12), 1581–1586. <https://doi.org/10.1038/ng.3703>
- James, K. N., Clark, M. M., Camp, B., Kint, C., Schols, P., Batalov, S., Briggs, B., Veeraraghavan, N., Chowdhury, S., & Kingsmore, S. F. (2020). Partially automated whole-genome sequencing reanalysis of previously undiagnosed pediatric patients can efficiently yield new diagnoses. *NPJ Genomic Medicine*, 5(1), 33. <https://doi.org/10.1038/s41525-020-00140-1>
- Johansen Taber, K. A., Dickinson, B. D., & Wilson, M. (2014). The promise and challenges of next-generation genome sequencing for clinical care. *JAMA Internal Medicine*, 174(2), 275–280. <https://doi.org/10.1001/jamainternmed.2013.12048>
- Kingsmore, S. F., Cakici, J. A., Clark, M. M., Gaughran, M., Feddock, M., Batalov, S., Bainbridge, M. N., Carroll, J., Caylor, S. A., Clarke, C., Ding, Y., Ellsworth, K., Farnaes, L., Hildreth, A., Hobbs, C., James, K., Kint, C. I., Lenberg, J., Nahas, S., ... RCI GM, I. (2019). A randomized, controlled trial of the analytic and diagnostic performance of singleton and trio, rapid genome and exome sequencing in ill infants. *American Journal of Human Genetics*, 105(4), 719–733. <https://doi.org/10.1016/j.ajhg.2019.08.009>
- Kingsmore, S. F., Henderson, A., Owen, M. J., Clark, M. M., Hansen, C., Dimmock, D., Chambers, C. D., Jelliffe-Pawlowski, L. L., & Hobbs, C. (2020). Measurement of genetic diseases as a cause of mortality in infants receiving whole genome sequencing. *NPJ Genomic Medicine*, 5(1), 49. <https://doi.org/10.1038/s41525-020-00155-8>
- Kingsmore, S. F., & Saunders, C. J. (2011). Deep sequencing of patient genomes for disease diagnosis: When will it become routine? *Science Translational Medicine*, 3(87), 87ps23. <https://doi.org/10.1126/scitranslmed.3002695>
- Köhler, S., Vasilevsky, N. A., Engelstad, M., Foster, E., McMurry, J., Aymé, S., Baynam, G., Bello, S. M., Boerkoel, C. F., Boycott, K. M., Brudno, M., Buske, O. J., Chinnery, P. F., Cipriani, V., Connell, L. E., Dawkins, H. J., DeMare, L. E., Devereau, A. D., de Vries, B. B., ... Robinson, P. N. (2017). The human phenotype ontology in 2017. *Nucleic Acids Research*, 45(D1), D865–D876. <https://doi.org/10.1093/nar/gkw1039>
- Kuehne, B., Heine, E., Dafsari, H. S., Irwin, R., Heller, R., Bangen, U., Brockmeier, K., Kribs, A., Oberthuer, A., & Cirak, S. (2019). Use of whole exome sequencing in the NICU: Case of an extremely low birth weight infant with syndromic features. *Molecular and Cellular Probes*, 45, 89–93. <https://doi.org/10.1016/j.mcp.2019.03.002>
- Landrum, M. J., Lee, J. M., Benson, M., Brown, G., Chao, C., Chitipiralla, S., & Maglott, D. R. (2016). ClinVar: Public archive of interpretations of clinically relevant variants. *Nucleic Acids Research*, 44, D862–D868.
- Li, H., & Durbin, R. (2010). Fast and accurate long-read alignment with Burrows-Wheeler transform. *Bioinformatics*, 26(5), 589–595. <https://doi.org/10.1093/bioinformatics/btp698>
- Li, Q., & Wang, K. (2017). InterVar: Clinical interpretation of genetic variants by the 2015 ACMG-AMP Guidelines. *American Journal of Human Genetics*, 100(2), 267–280. <https://doi.org/10.1016/j.ajhg.2017.01.004>
- McAllister, D. A., Liu, L., Shi, T., Chu, Y., Reed, C., Burrows, J., Adey, D., Rudan, I., Black, R. E., Campbell, H., & Nair, H. (2019). Global, regional, and national estimates of pneumonia morbidity and mortality in children younger than 5 years between 2000 and 2015: A systematic analysis. *Lancet Glob Health*, 7(1), e47–e57. [https://doi.org/10.1016/S2214-109X\(18\)30408-X](https://doi.org/10.1016/S2214-109X(18)30408-X)
- Meng, L., Pammi, M., Saronwala, A., Magoulas, P., Ghazi, A. R., Vetrini, F., Zhang, J., He, W., Dharmadhikari, A. V., Qu, C., Ward, P., Braxton, A., Narayanan, S., Ge, X., Tokita, M. J., Santiago-Sim, T., Dai, H., Chiang, T., Smith, H., ... Lalani, S. R. (2017). Use of exome sequencing for infants in intensive care units: Ascertainment of severe single-gene disorders and effect on medical management. *JAMA Pediatrics*, 171(12), e173438. <https://doi.org/10.1001/jamapediatrics.2017.3438>
- Mestek-Boukhibar, L., Clement, E., Jones, W. D., Drury, S., Ocaka, L., Gagunashvili, A., Le Quesne Stabej, P., Bacchelli, C., Jani, N., Rahman, S., Jenkins, L., Hurst, J. A., Bitner-Glindzicz, M., Peters, M., Beales, P. L., & Williams, H. J. (2018). Rapid Paediatric Sequencing (RaPS): Comprehensive real-life workflow for rapid diagnosis of

- critically ill children. *Journal of Medical Genetics*, 55(11), 721–728. <https://doi.org/10.1136/jmedgenet-2018-105396>
- Ng, B. G., Sharma, V., Sun, L., Loh, E., Hong, W., Tay, S. K. H., & Freeze, H. H. (2011). Identification of the first COG-CDG patient of Indian origin. *Molecular Genetics and Metabolism*, 102(3), 364–367. <https://doi.org/10.1016/j.ymgme.2010.11.161>
- OMIM. <https://www.omim.org/wlnter>
- Orphanet. <https://www.orpha.net/consor/cgi-bin/index.php>
- Petrikina, J. E., Cakici, J. A., Clark, M. M., Willig, L. K., Sweeney, N. M., Farrow, E. G., Saunders, C. J., Thiffault, I., Miller, N. A., Zellmer, L., Herd, S. M., Holmes, A. M., Batalov, S., Veeraraghavan, N., Smith, L. D., Dimmock, D. P., Leeder, J. S., & Kingsmore, S. F. (2018). The NSIGHT1-randomized controlled trial: Rapid whole-genome sequencing for accelerated etiologic diagnosis in critically ill infants. *NPJ Genomic Medicine*, 3, 6. <https://doi.org/10.1038/s41525-018-0045-8>
- Pinxten, W., & Howard, H. C. (2014). Ethical issues raised by whole genome sequencing. *Best Practice and Research. Clinical Gastroenterology*, 28(2), 269–279. <https://doi.org/10.1016/j.bpg.2014.02.004>
- Primer-BLAST. <https://www.ncbi.nlm.nih.gov/tools/primer-blast/>
- Rehm, H. L., Berg, J. S., Brooks, L. D., Bustamante, C. D., Evans, J. P., Landrum, M. J., Ledbetter, D. H., Maglott, D. R., Martin, C. L., Nussbaum, R. L., Plon, S. E., Ramos, E. M., Sherry, S. T., Watson, M. S., & ClinGen. (2015). ClinGen--The clinical genome resource. *New England Journal of Medicine*, 372(23), 2235–2242. <https://doi.org/10.1056/NEJMs1406261>
- Rentzsch, P., Witten, D., Cooper, G. M., Shendure, J., & Kircher, M. (2019). CADD: Predicting the deleteriousness of variants throughout the human genome. *Nucleic Acids Research*, 47(D1), D886–d894. <https://doi.org/10.1093/nar/gky1016>
- Reynders, E., Foulquier, F., Leão Teles, E., Quelhas, D., Morelle, W., Rabouille, C., Annaert, W., & Matthijs, G. (2009). Golgi function and dysfunction in the first COG4-deficient CDG type II patient. *Human Molecular Genetics*, 18(17), 3244–3256. <https://doi.org/10.1093/hmg/ddp262>
- Richards, S., Aziz, N., Bale, S., Bick, D., Das, S., Gastier-Foster, J., Grody, W. W., Hegde, M., Lyon, E., Spector, E., Voelkerding, K., Rehm, H. L., & ACMG Laboratory Quality Assurance, C. (2015). Standards and guidelines for the interpretation of sequence variants: A joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genetics in Medicine*, 17(5), 405–424. <https://doi.org/10.1038/gim.2015.30>
- Ritter, A., Bedoukian, E., Berger, J. H., Copenheaver, D., Gray, C., Krantz, I., Izumi, K., Juusola, J., Leonard, J., Lin, K., Medne, L., Santani, A., Skraban, C., Yang, S., & Ahrens-Nicklas, R. C. (2020). Clinical utility of exome sequencing in infantile heart failure. *Genetics in Medicine*, 22(2), 423–426. <https://doi.org/10.1038/s41436-019-0654-3>
- Sanford, E. F., Clark, M. M., Farnaes, L., Williams, M. R., Perry, J. C., Ingulli, E. G., Sweeney, N. M., Doshi, A., Gold, J. J., Briggs, B., Bainbridge, M. N., Feddock, M., Watkins, K., Chowdhury, S., Nahas, S. A., Dimmock, D. P., Kingsmore, S. F., Coufal, N. G., & RCIGM, I. (2019). Rapid whole genome sequencing has clinical utility in children in the PICU. *Pediatric Critical Care Medicine*, 20(11), 1007–1020. <https://doi.org/10.1097/PCC.0000000000002056>
- Schofield, D., Rynehart, L., Shrestha, R., White, S. M., & Stark, Z. (2019). Long-term economic impacts of exome sequencing for suspected monogenic disorders: Diagnosis, management, and reproductive outcomes. *Genetics in Medicine*, 21(11), 2586–2593. <https://doi.org/10.1038/s41436-019-0534-x>
- Smith, E. D., Blanco, K., Sajan, S. A., Hunter, J. M., Shinde, D. N., Wayburn, B., Rossi, M., Huang, J., Stevens, C. A., Muss, C., Alcaraz, W., Hagman, K., Tang, S., & Radtke, K. (2019). A retrospective review of multiple findings in diagnostic exome sequencing: Half are distinct and half are overlapping diagnoses. *Genetics in Medicine*, 21(10), 2199–2207. <https://doi.org/10.1038/s41436-019-0477-2>
- Smith, L. D., Willig, L. K., & Kingsmore, S. F. (2015). Whole-exome sequencing and whole-genome sequencing in critically ill neonates suspected to have single-gene disorders. *Cold Spring Harbor Perspectives in Medicine*, 6(2), a023168. <https://doi.org/10.1101/cshperspect.a023168>
- Soden, S. E., Saunders, C. J., Willig, L. K., Farrow, E. G., Smith, L. D., Petrikina, J. E., Lepichon, J. B., Miller, N. A., Thiffault, I., Dinwiddie, D. L., Twist, G., Noll, A., Heese, B. A., Zellmer, L., Atherton, A. M., Abdelmoity, A. T., Safina, N., Nyp, S. S., Zuccarelli, B., ... Kingsmore, S. F. (2014). Effectiveness of exome and genome sequencing guided by acuity of illness for diagnosis of neurodevelopmental disorders. *Science Translational Medicine*, 6(265), 265ra168. <https://doi.org/10.1126/scitranslmed.3010076>
- Stark, Z., Schofield, D., Alam, K., Wilson, W., Mupfeki, N., Macciocia, I., Shrestha, R., White, S. M., & Gaff, C. (2017). Prospective comparison of the cost-effectiveness of clinical whole-exome sequencing with that of usual care overwhelmingly supports early use and reimbursement. *Genetics in Medicine*, 19(8), 867–874. <https://doi.org/10.1038/gim.2016.221>
- Stark, Z., Schofield, D., Martyn, M., Rynehart, L., Shrestha, R., Alam, K., Lunke, S., Tan, T. Y., Gaff, C. L., & White, S. M. (2019). Does genomic sequencing early in the diagnostic trajectory make a difference? A follow-up study of clinical outcomes and cost-effectiveness. *Genetics in Medicine*, 21(1), 173–180. <https://doi.org/10.1038/s41436-018-0006-8>
- Stark, Z., Tan, T. Y., Chong, B., Brett, G. R., Yap, P., Walsh, M., Yeung, A., Peters, H., Mordaunt, D., Cowie, S., Amor, D. J., Savarirayan, R., Mcivillray, G., Downie, L., Ekert, P. G., Theda, C., James, P. A., Yaplitto-Lee, J., Rya, M. M., ... White, S. M. (2016). A prospective evaluation of whole-exome sequencing as a first-tier molecular test in infants with suspected monogenic disorders. *Genet Med*, 18(11), 1090–1096.
- Stenson, P. D., Mort, M., Ball, E. V., Chapman, M., Evans, K., Azevedo, L., & Cooper, D. N. (2020). The Human Gene Mutation Database (HGMD (®)): Optimizing its use in a clinical diagnostic or research setting. *Human Genetics*, 139, 1197–1207. <https://doi.org/10.1007/s00439-020-02199-3>
- Talevich, E., Shain, A. H., Botton, T., & Bastian, B. C. (2016). CNVkit: Genome-wide copy number detection and visualization from targeted DNA sequencing. *PLoS Computational Biology*, 12(4), e1004873. <https://doi.org/10.1371/journal.pcbi.1004873>
- Tan, N. B., Tan, T. Y., Martyn, M. M., Savarirayan, R., Amor, D. J., Moody, A., White, S. M., & Stark, Z. (2019). Diagnostic and service impact of genomic testing technologies in a neonatal intensive care unit. *Journal of Paediatrics and Child Health*, 55(11), 1309–1314. <https://doi.org/10.1111/jpc.14398>
- Tan, T. Y., Dillon, O. J., Stark, Z., Schofield, D., Alam, K., Shrestha, R., Chong, B., Phelan, D., Brett, G. R., Creed, E., Jarmolowicz, A., Yap, P., Walsh, M., Downie, L., Amor, D. J., Savarirayan, R., McGivillray, G., Yeung, A., Peters, H., ... White, S. M. (2017). Diagnostic impact and cost-effectiveness of whole-exome sequencing for ambulant children with suspected monogenic conditions. *JAMA Pediatr*, 171(9), 855–862. <https://doi.org/10.1001/jamapediatrics.2017.1755>
- Thorvaldsdottir, H., Robinson, J. T., & Mesirov, J. P. (2013). Integrative Genomics Viewer (IGV): High-performance genomics data visualization and exploration. *Briefings in Bioinformatics*, 14(2), 178–192. <https://doi.org/10.1093/bib/bbs017>
- Trower, M. K., & Elgar, G. S. (1994). PCR cloning using T-vectors. *Methods in Molecular Biology*, 31, 19–33. <https://doi.org/10.1385/0-89603-258-2-19>

UCSC. <https://genome.ucsc.edu/>

- van Diemen, C. C., Kerstjens-Frederikse, W. S., Bergman, K. A., de Koning, T. J., Sikkema-Raddatz, B., van der Velde, J. K., Abbott, K. M., Herkert, J. C., Löhner, K., Rump, P., Meems-Veldhuis, M. T., Neerinx, P., Jongbloed, J., van Ravenswaaij-Arts, C. M., Swertz, M. A., Sinke, R. J., van Langen, I. M., & Wijmenga, C. (2017). Rapid targeted genomics in critically ill newborns. *Pediatrics*, *140*(4), <https://doi.org/10.1542/peds.2016-2854>
- Var. <https://wintervar.wglab.org/>
- Varsome. <https://varsome.com/>
- Vulliamy, T., Beutler, E., & Luzzatto, L. (1993). Variants of glucose-6-phosphate dehydrogenase are due to missense mutations spread throughout the coding region of the gene. *Human Mutation*, *2*(3), 159–167.
- Wang, H., Lu, Y., Dong, X., Lu, G., Cheng, G., Qian, Y., Ni, Q., Zhang, P., Yang, L., Wu, B., & Zhou, W. (2020). Optimized trio genome sequencing (OTGS) as a first-tier genetic test in critically ill infants: Practice in China. *Human Genetics*, *139*(4), 473–482. <https://doi.org/10.1007/s00439-019-02103-8>
- Wang, H., Qian, Y., Lu, Y., Qin, Q., Lu, G., Cheng, G., Zhang, P., Yang, L., Wu, B., & Zhou, W. (2020). Clinical utility of 24-h rapid trio-exome sequencing for critically ill infants. *NPJ Genomic Medicine*, *5*, 20. <https://doi.org/10.1038/s41525-020-0129-0>
- Wang, K., Li, M., & Hakonarson, H. (2010). ANNOVAR: Functional annotation of genetic variants from high-throughput sequencing data. *Nucleic Acids Research*, *38*(16), e164. <https://doi.org/10.1093/nar/gkq603>
- Watson, R. S., & Carcillo, J. A. (2005). Scope and epidemiology of pediatric sepsis. *Pediatric Critical Care Medicine*, *6*(3 Suppl), S3–S5. <https://doi.org/10.1097/01.PCC.0000161289.22464.C3>
- Weiner, J., Sharma, J., Lantos, J., & Kilbride, H. (2011). How infants die in the neonatal intensive care unit: Trends from 1999 through 2008. *Archives of Pediatrics and Adolescent Medicine*, *165*(7), 630–634. <https://doi.org/10.1001/archpediatrics.2011.102>
- Willig, L. K., Petrikin, J. E., Smith, L. D., Saunders, C. J., Thiffault, I., Miller, N. A., Soden, S. E., Cakici, J. A., Herd, S. M., Twist, G., Noll, A., Creed, M., Alba, P. M., Carpenter, S. L., Clements, M. A., Fischer, R. T., Hays, J. A., Kilbride, H., McDonough, R. J., ... Kingsmore, S. F. (2015). Whole-genome sequencing for identification of Mendelian disorders in critically ill infants: A retrospective analysis of diagnostic and clinical findings. *The Lancet Respiratory Medicine*, *3*(5), 377–387. [https://doi.org/10.1016/s2213-2600\(15\)00139-3](https://doi.org/10.1016/s2213-2600(15)00139-3)
- Wright, C. F., FitzPatrick, D. R., & Firth, H. V. (2018). Paediatric genomics: Diagnosing rare disease in children. *Nature Reviews Genetics*, *19*, 253–268. <https://doi.org/10.1038/nrg.2017.116>
- Wu, E. T., Hwu, W. L., Chien, Y. H., Hsu, C., Chen, T. F., Chen, N. Q., Chou, H. C., Tsao, P. N., Fan, P. C., Tsai, I. J., Lin, S. P., Hsieh, W. S., Chang, T. M., Chen, C. N., Lee, C. H., Chou, Y. Y., Chiu, P. C., Tsai, W. H., Hsiung, H. C., ... Lee, N. C. (2019). Critical trio exome benefits in-time decision-making for pediatric patients with severe illnesses. *Pediatric Critical Care Medicine*, *20*(11), 1021–1026. <https://doi.org/10.1097/PCC.0000000000002068>
- Yang, H., Robinson, P. N., & Wang, K. (2015). Phenolyzer: Phenotype-based prioritization of candidate genes for human diseases. *Nature Methods*, *12*(9), 841–843. <https://doi.org/10.1038/nmeth.3484>
- Yang, L., Liu, X., Li, Z., Zhang, P., Wu, B., Wang, H., Hu, L., Cheng, G., Wang, L., & Zhou, W. (2020). Genetic aetiology of early infant deaths in a neonatal intensive care unit. *Journal of Medical Genetics*, *57*(3), 169–177. <https://doi.org/10.1136/jmedgenet-2019-106221>
- Ye, J., Coulouris, G., Zaretskaya, I., Cutcutache, I., Rozen, S., & Madden, T. L. (2012). Primer-BLAST: A tool to design target-specific primers for polymerase chain reaction. *BMC Bioinformatics*, *13*, 134. <https://doi.org/10.1186/1471-2105-13-134>
- Zhu, C. P., Wu, X. H., Liang, Y. T., Ma, W. C., & Ren, L. (2015). The mortality of patients in a pediatric emergency department at a tertiary medical center in China: An observational study. *World Journal of Emergency Medicine*, *6*(3), 212–216. <https://doi.org/10.5847/wjem.j.1920-8642.2015.03.009>

## SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

**How to cite this article:** Liu, J., Zheng, Y., Huang, J., Zhu, D., Zang, P., Luo, Z., Yang, Y., Peng, Y., Xiao, Z., Zhu, Y., & Lu, X. (2021). Expanding the genotypes and phenotypes for 19 rare diseases by exome sequencing performed in pediatric intensive care unit. *Human Mutation*, *42*, 1443–1460. <https://doi.org/10.1002/humu.24266>