Clinical and Molecular Findings in Early Infant Death Associated with Genetic Diseases: A Multicenter Cohort Study in China

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Research

Abstract

Background To investigate the impact of exome sequencing in critically ill infants and find out high-risk patients with genetic diseases for the 180-day mortality rate.

Results A molecular diagnosis rate achieved in 203 of 439 (46.2%) sequenced infants. 60 of 203 (29.5%) diagnosed patients died before 180 days of life. Compared to the undiagnosed-cases, patients with genetic findings in metabolic-endocrine disorders (OR=2.89, 95% CI: 1.51-5.52), primary immunodeficiencies (OR=3.79, 95% CI: 1.25-11.53), and early-onset muscle disease (OR=6.49, 95% CI: 2.28-18.48) had high odds ratios of 180-day mortality. Based on the functions of the defective genes, infants with catalytic activity deficiency (OR=3.07, 95% CI: 1.51-6.25) and transporter activity deficiency (OR=4.15, 95% CI: 1.80-9.56) also had a high risk of 180-day mortality. Furthermore, the proportion of medical management correspondence to genetic findings increased from 22.4% to 40.3% (*p*=0.06), and the 180-day mortality rate decreased from 35.9% to 29.6% showing a moderate decreased trend (*p* trend =0.08) over 4 years.

Conclusions Our study shows that patients with genetic findings in metabolic-endocrine disorders, primary immunodeficiencies and early-onset muscle disease are related to 180-day mortality, which are of great significance for improve the prognosis.

Background

Genetic diseases are common among infants in neonatal intensive care units (NICUs), with an incidence of 16–20% [1–3]. Disease progression can be very rapid, and a significant proportion of the affected individuals succumb to their diseases early in life[4]. Early preventive measures implemented for decreasing genetic diseases mortality would reduce mortality rate under five years of age significantly [5, 6].

The clinical manifestations of more than 14,000 genetic diseases may be atypical and overlapped[4, 7]. The prognosis and complications of each genetic disease are variable. Previous studies usually compared the mortality risk based on phenotypic system, chromosome disorder categories, recognized or unrecognized syndromes, and single or multiple defects[8, 9]. Because the patients' symptoms are overlapped and may not be fully developed or recognized in young infants, the phenotypic category system would lead to an under-estimated[10]. Fortunately, the clinical application of next-generation sequencing empowers us to detect genetic etiology efficiently. Cause-specific mortality classification of the underlying cause of death will be necessary for public health interventions to etiology-specific populations, including limited diagnostic resources [11–13]. Currently, there are few published studies comparing the odds ratio of infantile mortality among categories of genetic disease according to phenotype related genes.

Here, we retrospectively examined the odds ratio for 180-day mortality in a total of 439 infants who underwent exome sequencing in our multicenter genetic cohort.

Results

Demographics of the study population

A total of 439 NICU infants were enrolled. There were 266 males (60.6%) and 173 females (39.4%), with a median (quartile) age of 7.0 (1.0, 26.0) days at the time of hospital admission. There were 49 (11.2%) preterm newborns (gestational week ≤ 32), with a median gestational age of 38.1 (35.9, 39.5) weeks. A total of 147 (33.5%) newborns were hospitalized on the first day of life. The median (days) (quartile) age at onset was 1.0 (1.0, 11.0), age at admission was 3.5 (1.0, 26.0), and NICU stay length was 16.0 (8.0, 30.0). 99 infants died before 180 days of life, with a median age of 55.0 (19.0, 109.0) days at death. The main symptoms leading to genetic testing included multiple malformation (162, 36.9%), seizures and hypotonia (158, 35.9%), metabolic crisis and endocrine disturbance (105, 23.9%), recurrent and severe infection (67, 15.3%), cardiac anomalies (24, 5.5%), and others (95, 21.6%). The clinical characteristics of the genetic cohort are shown in Supplement Table 1.

Table 1
Summary of exome sequencing and medical management in the enrolled infants.

Characteristic	N (%) Median (quartiles)	2016-2017[a]	2018	2019	P value [b][c]
N	439	125	154	160	NA
Exome sequencing					0.23
TES	83	21	25	37	
WES	356	104	129	123	
Sequencing type					0.33
Singleton	84	29	25	30	
Trios	355	96	129	130	
Age at genetic testing (days)	19.0 (8.0, 41.0)	23.0 (10.0, 42.0)	18.0 (9.0, 33.0)	15.0 (6.0, 45.0)	0.36
Time to genetic diagnosis (days)					
Admission to attainment of parents' consent	6.0 (3.0, 12.0)	6.0 (2.0, 11.0)	7.0 (3.0,16.0)	6.0 (3.0, 9.5)	0.15
Sample receipt to report	46.0 (33.0, 66.0)	64.0 (45.0, 66.0)	48.0 (35.0, 66.0)	28.0(24.0, 36.0)	< 0.001
Molecular positivity rate					0.82
Diagnosed	203 (46.2)	58 (46.4)	68 (44.2)	77 (48.1)	
Undiagnosed	236 (53.8)	67 (53.6)	86 (55.8)	83 (51.9)	
Medical management					NA
Redirection of care	12	3	5	4	

TES: Targeted exome sequencing; WES: Whole exome sequencing. NA, not applicable.
Categorical variables are presented as numbers (%).
Continuous variables are presented as medians (quartiles).
[a] Merged data from 2016 and 2017 due to a moderate sample size.
[b] 2-Tailed *t* test or Fisher exact test, when applicable.
[c] Comparation among three periods, 2016−2017, 2018 and 2019.

Characteristic	N (%) Median (quartiles)	2016-2017[a]	2018	2019	*P* value [b c]
Initiation of new subspecialist care	35	7	13	15	
Changes in medication or diet	18	3	6	9	
Major procedures	5	0	2	3	
Total proportion in diagnosed cases (%)	70 (34.5)	13 (22.4)	26 (38.2)	31 (40.3)	0.06
180-day mortality in diagnosed infants					0.31
Deceased	60 (29.5)	21 (35.9)	21 (30.9)	18 (29.6)	
Survived	143 (70.5)	38 (64.41)	47 (69.1)	58 (70.4)	
P trend of 180-day mortality across years					0.08
TES: Targeted exome sequencing; WES: Whole exome sequencing. NA, not applicable.					
Categorical variables are presented as numbers (%).					
Continuous variables are presented as medians (quartiles).					
[a] Merged data from 2016 and 2017 due to a moderate sample size.					
[b] 2-Tailed *t* test or Fisher exact test, when applicable.					
[c] Comparation among three periods, 2016–2017, 2018 and 2019.					

Diagnostic And Turnaround Time Of Exome Sequencing

The number of enrolled infants suspected of genetic diseases was 30, 95, 154, and 160 in 2016, 2017, 2018, and 2019, respectively. Due to the moderate sample size, we merged the 2016 and 2017 data. The genetic testing methods included singleton (n = 84, 19.1%), trios (n = 355, 80.9%), TES (n = 83, 18.9%), and WES (n = 356, 81.1%), depending on the availability of parental samples and the suggestion of our multidisciplinary team. The molecular diagnosis rate was 59 (47.2%), 68 (44.2%), and 76 (47.5%) over three periods, without a significant difference. The time from patient admission to the attainment of the parents' consent remained unchanged. However, the median days (quartiles) from sample receipt to report (positive and/or negative) was respectively 64.0 days (45.0, 66.0), 48.0 days (35.0, 66.0), and 28.0 days (24.0, 36.0) across 2016–2017, 2018 and 2019 with significant difference ($p < 0.001$) (Table 1).

Genetic Disease Spectrum Of The Diagnosed Infants

Of the 439 infants, 203 (46.2%) were molecularly diagnosed with 143 disorders. Autosomal dominant, autosomal recessive, and X-linked disorders were observed in 93 (45.8%), 83 (40.9%), and 28 (13.8%) infants, respectively. Of the genetically diagnosed cases, 178 (87.7%) infants had monogenic disorders. Although 23 genes were hit multiple times, we found that 120 (59.1%) genes were hit only once, reflecting genetic heterogeneity in NICU infants. Among the monogenic disorders, familial hyperinsulinemia hypoglycemia (OMIM 256450), Kabuki syndrome (OMIM 147920), methylmalonic aciduria and homocystinuria (OMIM 277400), ornithine trans-carboxylase deficiency (OMIM 311250), inflammatory bowel disease 28 (OMIM 613148), and spinal muscular atrophy (OMIM 253300), caused by variants in *ABCC8, KMT2D, MMACHC, OTC, IL-10RA*, and *SMN1*, respectively, were the most frequent monogenetic diseases and were observed in 24 infants in the cohort. 25 (25/203, 12.3%) infants had CNVs, including three aneuploidy variants and 22 microdeletion/duplication variants. The recurrent causative aberrations were Prader-Willi Syndrome (28.0%), 16p11.2p12.2 deletion (12.0%), Jacobsen syndrome (8.0%), and Xp11.23-p11.22 duplication (8.0%) (Supplement Table 2).

Table 2
Comparison of 180-day mortality rate across eight categories based on phenotype-related genes.

Category	Total number	180-day mortality (%)	OR (95% CI)	Phenotype-related genes
G0	236	39 (16.5)	Ref.	/
G1	55	20 (36.4)	2.89 (1.51,5.52) **	*ABCC8; ACADVL; ALG12; ALPL; BSCL2; CASR; CPS1; CYP21A2; DUOX2; FOXP3; G6PD; GNAS; GSS; KCNJ11; MARS; MMACHC; MUT; NPC1; OTC; PAX8; PCSK1; PEX1; PEX26; PKLR; PRKAR1A; SCNN1A; SCNN1G; SLC6A19; STAR; TBX19; TRPM6; UGT1A1;*
G2	36	9 (25.0)	1.68 (0.74, 3.86)	*11q24.1-25 del; 12p13.33-11.21 dup; 15q11.2-13.1 del; 16p11.2-p12.2 del; 16q Trisomy; 18 Trisomy; 19p13.12 del; 21q22.12-22.13 del; 7q11.23 del; ATP6V1B2; BRAF; CREBBP; DDX58; EFTUD2; HRAS; KMT2D; NIPBL; NOTCH1; PTPN11; SHOC2;*
G3	29	6 (20.7)	1.32 (0.50, 3.45)	*20q13.33 del; 2q37.3 del; 9q33.3 del; AP4E1; AP4S1; ATN1; CHRNA2; FGFR2; FOXP1; GLDC;KAT6B; KCNQ2; MECP2; NPRL2; PACS2; POLE; PPP2R5D; PUM1; SCN2A; SCN3A; SCN8A; SMO; STAG2; TSC2; UBA5; Xp11.23-p11.22 dup; ZC4H2*
G4	16	9 (56.3)	6.49 (2.28, 18.48) **	*CHRNA1; DMD; GTPBP3; IGHMBP2; MTM1; MYBPC3; MYH7; SMN1*
G5	15	1 (6.7)	0.36 (0.05, 2.82)	*ALOX12B;COL17A1;COL7A1; EDA; IKBKG; KPT9; KRT14; TGM1;*
G6	14	6 (42.9)	3.79 (1.25, 11.53) **	*22q11.2 del; CXCR4; CYBB; IKBKG; IL10RA; IL2RG; ITGB2;JAK3; PRF1; SPINK5;WASP*
G7	38	9 (23.7)	1.56 (0.68, 3.57)	**Hematological**: *F13A1; FANCC; FLI1; GP1BA; HBG2; RPL5; RPS19; TUBB1; UNC13D;* **Cardiac**: *CHD7; FBN2; RAF1; TGFBR2;* **Gastrointestinal**: *EPCAM; JAG1; MYO5B;* **Skeletal**: *AMER1; CLCN7; COL2A1; PLOD1;* **Nephrological**: *ATP6V0A4; AQP2; AVPR2; NPHS1;* **Respiratory**: *ABCA3; DNAH11; DNAH5;* **Vascular**: *FGFR3; RASA1* ; **Lymphatic**: *EPHB4; KIF11;* **Eyes**: *EYA1;* **Development Disorders**: *EZH2.*

G0: undiagnosed; G1: metabolic and endocrine disorders; G2: multiple congenital malformation; G3: neurological disorder; G4: early-onset muscle disease; G5: dermatological disorder; G6: primary immunodeficiencies; G7: other, including cases involving the following systems: hematological, cardiac; gastrointestinal, skeletal, nephrological, respiratory, vascular, lymphatic, eyes and development disorders.

**: $P < 0.01$.

Comparison of 180-day mortality rates across eight categories according to phenotype-related genes

Among the 203 diagnosed infants, 60 infants died before 180 days. 25 infants died prior to the genetic diagnosis. The 180-day mortality rate in the diagnosed infants was 18/59 (30.5%) in 2016–2017, 21/68 (30.9%), and 21/76 (27.6%), respectively. This represents a moderate decreasing trend (p trend = 0.08) (Table 1).

The 180-day mortality rates across the eight categories were as follows: G0 (39/236, 16.5%); G1 (20/55, 36.4%); G2 (9/36, 25.0%); G3 (6/29, 20.7%); G4 (9/16, 56.3%); G5 (1/15, 6.7%); G6 (6/14, 42.9%); and G7, other (9/38, 23.7%), involving systems including hematological (1/9, 14.3%), cardiac (0/6), gastrointestinal (2/6, 33.3%), skeletal (1/4, 205.0%), nephrological (1/4, 25.0%), respiratory (3/3, 100.0%), vascular (0/2), lymphatic (0/2), eyes (0/1) and development disorders (0/1).

When observing the odds ratios for obtaining the odds ratios of 180-day mortality, we set the undiagnosed G0 infants as the reference group. The G1 infants with metabolic and endocrine disorders (OR = 2.89, 95% CI: 1.51–5.52), G4 infants with early-onset muscle disease (OR = 6.49, 95% CI: 2.28–18.48), and G6 infants with primary immunodeficiencies (OR = 3.79, 95% CI: 1.25–11.53) had significantly high odds ratios of 180-day mortality rate. However, infants diagnosed with the category of G2, G3, G5 or G7 were not related to 180-day mortality (Table 2). Furthermore, Kaplan-Meier curves showed that the survival proportion of patients with G3, G5, and G7 categories shown the same trend as that of G0 infants (Fig. 1a). In contrast, the mortality rate of patients with G1, G4 and G6 presented significantly lower survival rates than G0 infants (< 60% survival) (Fig. 1b).

Comparison of 180-day mortality rates across six molecular function categories

Among the diagnosed infants with monogenic disorder, the 180-day mortality rates across six molecular function categories were as follows: M0, undiagnosed (38/235, 16.17%); M1, catalytic activity deficiency (16/43, 37.2%); M2: signaling pathway associated activity deficiency (12/45, 26.7%); M3 transporter activity deficiency (12/27, 44.4%); M4, DNA/RNA metabolism activity deficiency (10/42, 23.8%); and M5, other (6/22, 27.3%). Compared with M0, the M1 (OR = 3.07, 95% CI: 1.51–6.25) and M3 (OR = 4.15, 95% CI: 1.80–9.56) categories were significantly related to the 180-day mortality rate. However, M2 (OR = 2.11, 95% CI: 0.99–4.38), M4 (OR = 1.41, 95% CI: 0.63–3.19) and M5 (OR = 1.95, 95% CI: 0.72–5.29) had low odds ratios for the 180-day mortality rate (Table 3).

Table 3
Comparison of 180-day mortality rate across five categories of molecular function.

Category	Total number	180-day mortality (%)	OR (95% CI)	Mutated gene products
M0	197	38 (16.2)	Ref.	/
M1	43	16 (37.2)	3.07 (1.51, 6.25) *	*ACADVL; ALG12; ALOX12B; ALPL; BSCL2; CASR; CPS1; CYP21A2; DUOX2; G6PD; GLDC; GNAS; GSS; MARS; MMACHC; MUT; NPC1; OTC; PAX8; PCSK1; PEX1; PEX26; PKLR; STAR; TBX19; TRPM6; UGT1A1;*
M2	45	12 (26.7)	2.11 (0.99,4.38)	*AMER1; BRAF; CXCR4; CYBB; EDA; EPHB4; F13A1; FGFR2; FGFR3; GTPBP3; HRAS; IGHMBP2; IKBKG; IL10RA; IL2RG; ITGB2; JAG1; JAK3; MTM1; NPRL2; PLOD1; PPP2R5D; PRF1; PRKAR1A; PTPN11; RAF1; RASA1; SHOC2; SMO; SPINK5; TGFBR2; TGM1; TSC2; UBA5;*
M3	27	12 (44.4)	4.15 (1.80, 9.56) *	*ABCA3; ABCC8; AQP2; ATP6V0A4; ATP6V1B2; AVPR2; CHRNA1; CHRNA2; KCNJ11; KCNQ2; MYO5B; NPHS1; SCN2A; SCN3A; SCN8A; SCNN1A; SCNN1G; SLC6A19;*
M4	42	10 (23.8)	1.41 (0.63, 3.19)	*ATN1; CHD7; CREBBP; DDX58; DMD; DNAH11; DNAH5; EFTUD2; EYA1; EZH2; FANCC; FLI1; FOXP1; FOXP3; KAT6B; KMT2D; KRT14; MECP2; NIPBL; NOTCH1; POLE; PUM1; RPL5; RPS19; SMN1; STAG2; TUBB1; WAS; ZC4H2;*
M5	22	6 (27.3)	1.95 (0.72, 5.29)	*AP4E1; AP4S1; CLCN7; COL17A1; COL2A1; COL7A1; EPCAM; FBN2; GP1BA; HBG2; KIF11; KRT9; MTM1; MYBPC3; MYH7; PACS2; UNC13D;*

M0: undiagnosed; M1: catalytic activity deficiency, defined as catalysis of a biochemical reaction at physiological temperatures. M2: signaling pathway-associated activity deficiency, defined as a process beginning with an active signal and ending when a cellular response has been triggered. M3: transporter activity deficiency, defined as the process in which a solute is transported across a lipid bilayer from one side of a membrane to the other. M4: DNA/RNA metabolism activity deficiency, defined as interacting selectively and non-covalently with any nucleic acid. M5: other.

*: $P < 0.05$

A: redirection of care; B: initiation of new subspecialist care;

C: changes in medication or diet; D: major procedures;

The red boxes indicate a change to palliative therapy, the orange boxes indicate the initiation of new subspecialist care, the yellow boxes indicate medication or diet modifications, and the green boxes indicate major procedures, such as transplants.

The Influence Of Medical Management On Genetic Findings

Among 203 genetic diagnosed infants, 70 of 203 (34.5%) patients were under specific management based on molecular diagnosis. The exceptions included the following: 25 (12.3%) infants died prior to genetic diagnosis. 41 (20.2%) infants with specific phenotypes have implemented surgical or medical treatments under the clinical diagnosis. The proportion of medical management corresponding to genetic diagnosis was 13 (22.0%), 26 (38.2%), and 31 (40.3%) in 2016–2017, 2018 and 2019. Moderate improvement was achieved over a 4-year period ($p = 0.06$).

Of these 4 categories, we observed that 12 (5.9%) patients with critical and serious conditions underwent redirection of care, including palliative care and withdrawal of life support, such as patient 401, who presented with mechanical ventilator-dependent respiratory failure, was found to have pathogenetic variants in *MTM1*, relating to myotubular myopathy (OMIM: 310400). These patients underwent a palliative trajectory and family genetic counsel. Second, 35 (17.2%) patients benefitted from the initiation of additional subspecialist evaluation, such as by neurology, endocrinology, cardiology and surgery specialty teams. For example, patient 434 diagnosed with homozygous loss of the *SMN1* exon 7 causes spinal muscular atrophy (OMIM: 253300) at 15 days of life were referred to participate in ongoing gene therapy (BP 39056). Patient 315 was a 5-day-old girl with severe hypercalcemia and hyperparathyroidism who was transferred for partial parathyroidectomy on 25 days of life, earlier than the standard surgical day, as sequence-data revealed two inherited pathogenic variants in *CASR*, diagnostic of CASR-related-hyperparathyroidism (OMIM: 239200). Third, 18 (8.9%) patients had a change in their medical status and/or diet. For example, patient 355, diagnosed with *NPC1*-related Niemann-Pick disease type C1 (OMIM: 257220), was treated with Migalastat beginning at 75 days of life, avoiding severe progressive hepatic dysfunction, psychomotor delay and multiple organ damage. Finally, major procedures, such as transplant, were implemented for 5 patients (2.5%) who are currently alive. Hematopoietic stem cell transplant was undertaken in 4 affected infants, 2 patients (patient 457 and 464) with an *IL-10RA* mutation that caused early-onset Inflammatory Bowel Disease 28 (OMIM: 613148), a patient 38 with *ITGB2* homozygous mutations causing leukocyte adhesion deficiency (OMIM: 116920), and a patient 475 with *WAS* mutations that contributed to Wiskott-aldrich syndrome (OMIM: 301000). Another infant (patient 374) had successfully undergone partial-liver transplantation due to complicated heterozygous mutations in the *UGT1A1* gene (OMIM: 606785) (Fig. 2).

Diagnostic findings in the diagnosed cases involving inherited pathogenic variants prompted us to address prenatal diagnosis. 73 families were referred for reproductive counseling.

Discussion

In our study, a molecular diagnosis achieved in 203 of 439 (46.2%) sequenced infants, in agreement with published rates of diagnosis of genetic diseases by exome sequencing[3, 14, 15]. Our study found that infants diagnosed with metabolic and endocrine disorders, early-onset muscle disease, and primary immunodeficiencies had a high risk of 180-day mortality. Furthermore, the 180-day mortality rate decreased over four years, most likely due to the decreased turnaround time and the improvement of medical management.

With extensive use of next-generation sequencing technology, the contribution of genetic diseases to early infant mortality has been reported to progressively increase. Wojcik et al [16] reported that the proportion of genetic diseases confirmed by genetic testing among 1-year infant deaths reached 22%, higher than previous reports. Yang et al [17] showed that infants genetic diagnosed as multiple congenital malformations was one-third of neonatal mortality. The molecular etiology of pediatric mortality is becoming clearer [4, 18, 19].

Our study found that the 180-day mortality rate were related to genetic diseases of metabolic-endocrine disorders, primary immunodeficiencies, and early-onset muscle disease, which were potentially curable and preventive[20–22]. Developing a rapid diagnostic method with a short turnaround time is an urgent need and has an important impact on clinical decision and management. From 2016–2017, 2018 to 2019, the median days (quartiles) of turnaround time in our cohort was 64.0 days (45.0, 66.0), 48.0 days (35.0, 66.0), and 28.0 days (24.0, 36.0), respectively, with significant difference (p < 0.001). Compared to that of published studies[1, 3, 14, 15, 23, 24] (Supplement Table 3), the success rate was low. In 2018, 4 of 6 infants diagnosed as primary immunodeficiencies died prior to genetic findings, missing the chance of implemented hematopoietic stem cells transplantation. However, a total of five genetic diagnosed patients with primary immunodeficiencies were referred to undergo transplant in 2019, 4 patients survived after 180 days of life and only one patient died during waiting for the donor. In recent years in China, the technology of the rapid genomic and/or exome sequencing is having a good start [25, 26]. The management correspondence to genetic findings also improved recently (p = 0.06). Gene therapies for early-onset muscle diseases are under development and their full costs are significant. However, some clinical trials are undergoing recently. We referred a weakness and hypotonia newborn patient molecular diagnosed as spinal muscular atrophy (SMA) at 15 days of life to participate in a trial of gene therapy (BP 39056) in 2019. But in 2016–2018, four patients diagnosed with SMA were reluctantly choose palliative trajectory and have short life expectancy in the absence of treatment. In further, newborn screening even earlier in utero by genetic diagnostic technology for severe congenital muscle diseases would also benefit the affected families. The shorted turnaround time and improvement of medical management contribute decreasing 180-day mortality trend (p for trend = 0.08) over 4 years.

Interestingly, our study found that multiple congenital malformations was not the leading cause of the 180-day mortality rate, which contrasted to previous studies [27, 28]. Several explanations for this difference are presented below. First, the definition of congenital malformation in our study was genetic diagnosis with a pathogenic molecular or cytogenetic aberration, which was different from the clinical diagnoses which was often based on abnormal facial features, limbs and organs. However, the structural anomalies may or may not be genetic[29, 30]. Second, the decreased mortality rate can be explained by the fact that termination of pregnancy is legal in China in the case of fetal congenital anomalies based on the interpretation of neonatal and infant mortality statistics[31, 32]. Finally, improved prenatal detection and early treatment would almost certainly result in longer survival for the affected patients.

Additionally, our study found that catalytic activity and transporter activity are likely to be life-threatening. First, the catalytic activity category relates to enzyme proteins, which are present in multiple biological

processes and are pivotal to health and disease[33]. The high-risk categories of metabolic and endocrine disorders and primary immunodeficiency are usually associated with catalytic activity deficiency. Second, it was reported that transporter activity is another molecular function that is essential for life. Transporter gene mutations in neonates often show an early onset and result in metabolic crises that can be life-threatening if not treated in a timely manner[34]. For example, twin brothers developed cardiac arrhythmia due to severe hyponatremia, hyperkalemia, and metabolic acidosis at 9 days of life. One sibling died of cardiac arrest shortly after NICU admission, while the other died within 5 days after discharge. The homozygous mutation c.1439 + 1G > C in the *SCNN1A* gene was confirmed by exome sequencing. Although the molecular function of DNA/RNA metabolism is also important for life, our results show that diagnosis with signal pathway-associated activity deficiency and DNA/RNA metabolism deficiency was not related to the 180-day mortality rate. Infants diagnosed with congenital malformations were mostly distributed into these two categories. The flat odds ratios of congenital malformation may be the reason for the lowered risk. The molecular function analysis in our study showed that monogenic diseases caused by a mutation in a single gene with a clear molecular pathogenesis are usually lethal, and these results provided new targets and clues for further gene therapy research.

A strength of our study is that our application enabled us to study a large patient population over more than four years, ensure a follow-up time of at least 180 days after birth to measure outcomes, and compare groups of patients according to phenotype-related genes. Importantly, we found that infants genetically diagnosed with metabolic-endocrine disorders, primary immunodeficiencies and early-onset muscle disease showed significant risk for 180-day mortality. This study has some limitations. It is the retrospective design, yet we draw conclusion based on completed medical history and follow-up information. Another limitation is that we performed exome sequencing in the diagnostic pathway for those clinically suspected of genetic diseases, including newborns and infants with structural changes and those who were difficult to diagnose with other modalities, which may fail to identify all of the pathogenic variants. However, exome sequencing has shown the highest testing efficiency[19]. Our genetics team also reanalyzed sequencing data, which were negatives for the undiagnosed cases. Additionally, to minimize differences in medical diagnosis and treatment level, all the infants were recruited from three specialized children's hospitals affiliated to Jiao Tong University Medicine School. To draw more general conclusions, a prospective study with a larger cohort will be required.

Conclusions

Knowledge of genetic disease categories on infantile mortality is of significance for integrating preventive strategies to effectively care for patients and their families. More prospective studies focused on etiology-specific causes of mortality in genetic diseases are warranted.

Methods

Genetic disease cohort

We performed a retrospective mortality analysis for the underlying genetic cause of death from three tertiary children's hospitals, including Xinhua Hospital, Shanghai Children's Hospital and Shanghai Children's Medical Center, affiliated to Shanghai Jiao Tong University School of Medicine. These three hospitals served as training centers for medical students, pediatric and nurses, and family practice residents.

The patient enrollment inclusion criteria were as follows as detailed previously[35]: (1) Affected infants with suspected genetic conditions were referred for exome sequencing by a multidisciplinary medical team consisting of the treating neonatologists, medical geneticists and laboratory specialists from January 2016 to December 2019. (2) Infants were admitted before 100 days of life. (3) Clinical assessments of 180-day mortality (yes/no) were completed. The exclusion criteria were as follows: (1) one or more biological parents refused to participate; and (2) death of infants due to an acquired condition not clearly related to their underlying genetic etiology (Fig. 1).

Exome sequencing including whole exome sequencing (WES) and target exome sequencing (TES), were performed after genetic consultation by the multi-disciplinary medical team and attainment of consent from the parents who had children with the following conditions:1) multiple congenital malformation; 2) seizures and/or hypotonia; 3) metabolic crisis or endocrine disturbance; 4) recurrent and severe infection; 5) cardiac anomaly; and 6) other combined conditions suspected to be associated with genetic diseases in the absence of a clinical diagnosis (Fig. 3).

Exome Sequencing

Peripheral blood leukocytes were obtained and genomic DNA was extracted according to standard instructions using a QIAamp Blood DNA Mini Kit (Qiagen GMBH, Hilden, Germany). The capture probes were those used in GenCap Custom Exome Enrichment Kits (MyGenostics, Beijing, China) and TruSight Rapid Capture Kits (Illumina, Inc., San Diego, CA, USA). And then sequenced by Illumina HiSeq 6000. Burrows-Wheeler Aligner (BWA, version 0.7.10) was used to align the reads to the human reference genome (GRCh37/hg19). Copy number variations (CNVs) and small variants were identified using Genome Analysis Toolkit (GATK) (4.0.10.1).

The pathogenicity of candidate variants was evaluated followed the variant interpretation and reporting guideline of the American College of Medical Genetics (ACMG)[36, 37]. Genetic diagnostic findings were reported if identified pathogenic variants or likely pathogenic variants in according to a classical inheritance pattern. A case was classified as undiagnosed if it was analyzed by both WES and genome chromosomal microarray (CMA) but remained undiagnosed. Potential CNVs identified by sequencing were further assessed with karyotype testing or CMA. Sanger sequencing was performed to validate SNVs.

Classification System

The enrolled infants were classified into eight categories according to the phenotype-related genes as follows: G0: undiagnosed, G1: metabolic and endocrine disorders, G2: multiple congenital malformation, G3: neurological disorder, G4: early-onset muscle disease, G5: dermatologic disorder, G6: primary immunodeficiency, and G7: other, meaning those who did not fall into the above categories, including those with defects in the systems of hematological, cardiac, gastrointestinal, skeletal, nephrological, respiratory, vascular, lymphatic, eyes and development disorders.

Second, infants diagnosed with a monogenic disorder were classified into six categories on the basis of the molecular function of the gene (http://geneontology.org) as follows[38]: M0: undiagnosed; M1: catalytic activity deficiency, defined as catalysis of a biochemical reaction at physiological temperatures; M2: signaling pathway associated activity deficiency, defined as a process beginning with an active signal and ending when a cellular response has been triggered; M3: transporter activity deficiency, defined as the process in which a solute is transported across a lipid bilayer from one side of a membrane to the other; M4: DNA/RNA metabolism activity deficiency, defined as interacting selectively and non-covalently with any nucleic acid; and M5: other, including those who did not fall into the above categories.

Genetic diagnosis provides medical treatment in amenable cases, and comfort care to reduce patients' suffering in incurable cases. To evaluate the medical management of genetics findings, we classified medical managements into four categories (1) redirection of care, (2) initiation of new subspecialist care, (3) changes in medicine or diet, and (4) major procedures, such as organ transplant. However, there are three categories of exceptions: (A) infants who were genetically diagnosed postmortem, (B) infants with congenital malformation who underwent surgical procedures prior to the return of genetics results, and (C): infants whose biochemical phenotypes suggested a specific metabolic disease and were administrated a specific diet and medicine with clinical diagnoses.

Statistical analysis

Continuous data are presented as medians and quartiles. Categorical data are presented as numbers and constituent ratios. Data were compared using the chi-square test, Fisher's exact test, and ANOVA. A p value of 0.05 was used as a significance threshold and was calculated using SAS 9.2.

Abbreviations

NICUs: Neonatal Intensive Care Units; WES: Whole Exome Sequencing; TES: Target Exome Sequencing; SNVs: Single Nucleotide Variants; CNVs: Copy Number Variations; ACMG: American College of Medical Genetics; CMA: Chromosomal Microarray; HSCs: Hematopoietic Stem Cells; NA: Not Applicable

Declarations

Ethics Statement

This study involving human participants was reviewed and approved by the Ethics Committee of Xin Hua Hospital Affiliated to Shanghai Jiao Tong University School of Medicine (Approval number: XHEC-D-2020-095).

Consent for publication

All presentations of case reports have consent for publication.

Availability of data and material

The datasets used during and/or analyzed during the current study are available from the corresponding author on request.

Competing interests

None of the authors has any conflict of interest to disclose.

Funding

Contributors

YZ conceptualized and designed the study. XW, TZ, XG and FB prepared an analytical plan, analyzed the data, and drafted the initial manuscript. LM, JS, GQ and JS collected the original data. YC and DZ provided advice on statistical analysis of data. JW and YS provided essential suggestions on genetic data analysis. YZ critically reviewed the manuscript for important intellectual content and interpreted the data and results.

Acknowledgments

References
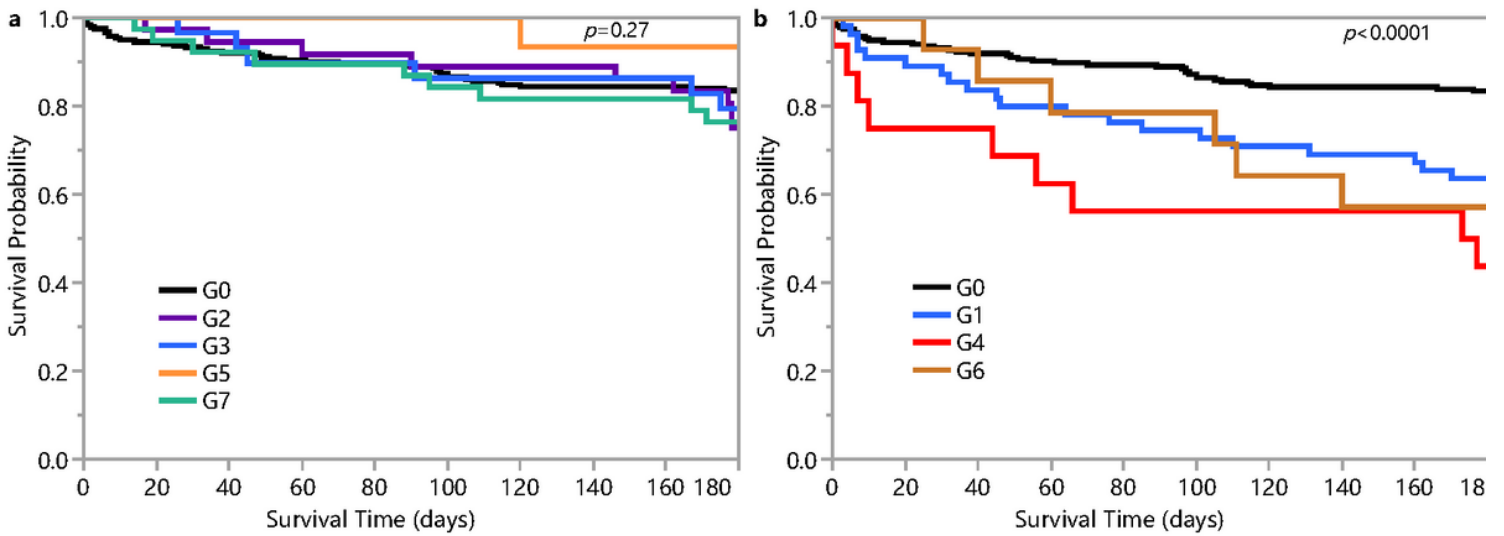
Figures

Figure 1

a. Kaplan-Meier curve showing the survival probability of the G0, G2, G3, G5, and G7 categories. b. Kaplan-Meier curve showing the survival probability of the G0, G1, G4, and G6 categories. G1: metabolic and endocrine disorders; G2: multiple congenital malformation; G3: neurological disorder; G4: early-onset muscle disease; G5: dermatological disorder; G6: primary immunodeficiencies; G7: others.

Figure 2

"See the Supplemental Files section for the complete figure caption".
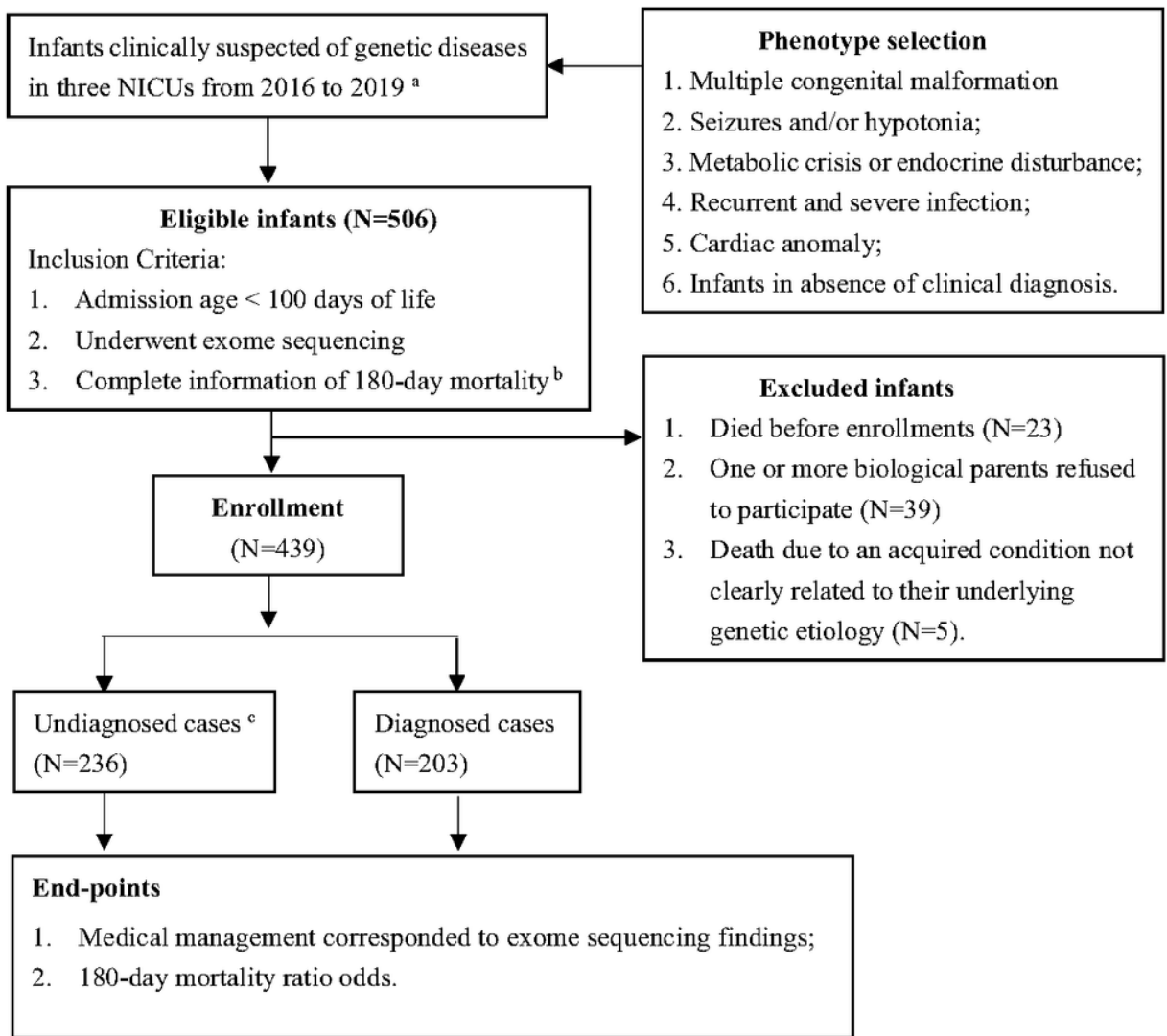
Figure 3

Flow diagram of study overview. a: "Infants clinically suspected of genetic diseases" refers to infants who were referred for exome sequencing by a multidisciplinary medical team consisting of the treating neonatologists, medical geneticists and laboratory specialists. b: 180-day mortality is defined as infants who died before 180 days of life. c: Undiagnosed cases defined as the infants analyzed by both WES and CMA but remained undiagnosed. And the undiagnosed cases were set as the reference group when calculating the 180-day mortality odds ratios of diagnosed cases.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Figure2Caption.docx

- SupplementTable1.docx
- SupplementTable2.xlsx
- SupplementTable3.docx