Supplementary Materials

Method

Sample collection and DNA extraction

Once pretest genetic counselling was done with informed consent obtained, the genetic counsellor or specialty nurse would liaise with the intensive care team for the timely collection of appropriate samples for DNA extraction. Where possible, trio samples (proband and biological parents) were obtained. While blood was the preferred sample type, if blood was not suitable (e.g. recent blood transfusion causing leukocyte contamination requiring a 2-week break prior to blood collection for DNA extraction), alternative sources such as skin biopsy for fibroblast culture and DNA extraction were done. Saliva samples and buccal swabs were deemed unsuitable for this study to yield a sufficient amount of DNA for testing,[1] as recruited children were often critically ill and intubated or unable to cooperate with spitting. Samples were then sent to the laboratory for RapidSeq. DNA was isolated using Gentra Puregene Blood Kit (Qiagen, Germantown, US) as per the manufacturer's protocol.

Sequencing, bioinformatic analysis and variant curation

Targeted sequencing of exonic regions of 4,813 genes associated with human diseases (also referred to as the clinical exome) was performed using the Illumina TruSight One sequencing panel (Illumina Inc, San Diego, US) and on the MiSeq Sequencer (Illumina, US). Variant calls were made using the MiSeq Reporter pipeline (Illumina, US) and aligned to human reference GRCh37/hg19. Variant annotation, filtration and prioritisation were performed using wANNOVAR[2] and Illumina Variant Studio (Illumina, US). Variants were checked against those in dbSNP,[3] 1000 Genomes,[4] Exome Aggregation Consortium (ExAC)[5] or Genome Aggregation Database (gnomAD),[6] ClinVar[7] and Human Gene Mutation Database (HGMD)[8] for information on frequencies and previously reported findings in patients.

Missense variants were further evaluated using in silico tools (SIFT,[9] Polyphen2[10] and Mutation Taster[11]) to predict the effect of the specific substitution on protein function.

Potential variants were reviewed in conjunction with the clinical genetics team. Where possible, we selected genes of interest (Supplementary Table S5) that correlated with the patient's phenotype for first pass analysis, and also used ClinVar (pathogenic/likely pathogenic variants) as an additional filter. If a genetic variant was identified to be consistent with the patient's primary phenotype, it was confirmed by Sanger sequencing in the index patients as well as parents, especially for those families where only proband DNA was sequenced, to determine the origin as well as phase of the variant(s). Variants were classified according to the published guidelines of the American College of Medical Genetics and Genomics (ACMG) and the Association of Molecular Pathology (AMP).[12] A clinical report was then generated, which was shared with the primary care team and the patient's family with a target turnaround time of 10 days.

Supplementary Table S1. Demographic and clinical characteristics

	Analysis method	**Age at recruitment**	**Sex**	**Referral source**	**Ethnicity**	**Primary system involved**
Case 1	Trio	7 days	Male	NICU	Chinese	Multiple
Case 2	Proband	4.5 months	Male	CICU	Chinese	Neurology
Case 3	Proband	3.6 months	Female	NICU	Chinese	Pulmonary
Case 4	Proband	12 days	Female	NICU	Chinese	Multiple
Case 5	Trio	NA	Female	NA	Chinese	Skeletal
Case 6	Proband	1.4 months	Female	NICU	Chinese	Multiple
Case 7	Trio	11 days	Female	NICU	Filipino/ Japanese	Multiple
Case 8	Trio	1.2 months	Male	NICU	Chinese	Pulmonary
Case 9	Trio	2 months	Male	CICU	Chinese	Multiple
Case 10	Trio	3 days	Male	NICU	Chinese	Multiple

Supplementary Table S2. Clinical outcomes

	Overall time from recruitment to result (days)	**Time from recruitment to sample collection (days)**	**Sample collection to DNA ready (days)**	**DNA ready to initiation of sequencing (days)**	**Sequencing (days)**	**Sequencing result ready for analysis to bioinformatic analysis (days)**	**Bioinformatic analysis to preliminary result (days)**	**Molecular diagnosis made**
Case 1	**7**	0	2	0	4	0	1	-
Case 2	**9**	0	1	2	4	2	0	*KIF1A*
Case 3	**12**	0	2	1	6	1	2	-
Case 4	**9**	0	1	2	5	1	0	-
Case 5	**13**	0	0	2	9	2	0	*IFT122*
Case 6	**19**	2	9	2	5	1	0	*SAMD9*
Case 7	**6**	0	1	0	4	1	0	-
Case 8	**10**	0	0	6	3	1	0	**FGFR3*
Case 9	**5**	0	1	0	4	0	0	-
Case 10	**10**	0	3	1	5	1	0	*SHOC2*
Median	**9.5**	0	1	1.5	4.5	1	0	

* Found on re-analysis 2 years later

Supplementary Table S3. Description of cases

Case 1 Case 1 was born preterm at 35+2 weeks gestation and referred to genetics at 5 days old for antenatally diagnosed hydrops fetalis with congenital chylothorax. Chromosomal microarray was sent and with no significant copy number variants found. He was recruited for RapidSeq for which results returned negative.

Case 2

Case 2 was born full term and was referred to genetics when he was admitted at 2 weeks old for *E. coli* right pyelonephritis and coronavirus infection complicated by recurrent apneas requiring intubation, and was noted to have subtle dysmorphic features with bilateral ear lidding, radial rotation of distal phalanx of right middle finger and contracted left index finger. A chromosomal microarray done was normal. He later readmitted at 4 months old for a breakthrough urinary tract infection and candida bronchopneumonia with norovirus gastroenteritis complicated by acute respiratory distress syndrome requiring extra corporeal membrane oxygenation support. He was then recruited for RapidSeq which revealed a de novo pathogenic mutation in *KIF1A* gene. Mutations in *KIF1A* gene are associated with autosomal dominant intellectual disability and peripheral neuropathy. Clinical features include microcephaly and distal arthrogryposis. Obtaining a diagnosis allowed the managing medical team a better understanding of the child, as well as enabled the appropriate surveillance for associated complications such as eye reviews for optic atrophy and visual impairment. It also enabled appropriate genetic counselling for family planning. The child later demised at 2 years 11 months old secondary to pneumonia.

Case 3

Case 3 was born full term at 39+2 weeks gestation. She was referred to genetics at day 2 of life for antenatally diagnosed hydrops fetalis requiring insertion of a thoraco-amniotic shunt and amnioreduction. Chromosomal karyotype showed 46 XX inv(9)(q32q34.3), while a chromosomal microarray found a deletion in 2q13 involving NPHP1 gene. Further testing was deferred at this point. However, at 2 months of age, the patient developed severe pulmonary hypertension for which the cause was unclear, hence RapidSeq was sent which returned negative. Further whole exome sequencing done under research also returned negative.

Case 4

Case 4 is a preterm infant born at 36 weeks who was referred to genetics at day 3 of life for postnatally diagnosed hydrops fetalis. She was also noted to have congenital shortening of the left middle finger. Chromosomal microarray did not find any significant copy number variants. RapidSeq was sent, which returned negative.

Case 5

Case 5 presented for prenatal genetic counselling while pregnant at 12 weeks of gestation. She had had a previous pregnancy which had been terminated as the fetus showed signs of skeletal dysplasia. No genetic diagnosis was made at that time, but the couple had been counselled that the risk of recurrence in subsequent pregnancies was low. However, an antenatal scan in the second pregnancy showed fetal anomalies similar to the first pregnancy.

Using a stored fetal DNA sample from the first pregnancy, the family was enrolled in RapidSeq. The team discovered pathogenic variants detected in *IFT122* gene, which is consistent with the diagnosis of cranioectodermal dysplasia. Cranioectodermal dysplasia is a multisystemic disorder causing skeletal dysplasia, ectodermal defects and joint laxity. Fewer than 60 cases have been reported in literature.

Case 6

Case 6 was born premature at 30 weeks of gestation and presented with hyperpigmentation of the skin. She was later found to have congenital adrenal insufficiency, with hypoplastic adrenal gland as well as severe persistent thrombocytopenia.

As the patient had received a packed cell and platelet transfusion 3 and 9 days respectively prior to recruitment, this delayed sample collection as in our institution, it is recommended to wait for 2 weeks following the last transfusion before collection of blood sample to prevent leukocyte contamination. As such, the patient underwent a skin biopsy for skin fibroblast culture and subsequent DNA extraction, adding an additional 11 days before DNA was ready for analysis.

The patient was later found to have a *de novo* known pathogenic mutation C>G in the *SAMD9* gene. Mutations in the *SAMD9* gene are associated with MIRAGE (Myelodysplasia, Infection, Restriction of growth, Adrenal hypoplasia, Genital phenotypes and enteropathy) syndrome, which is consistent with the patient's clinical presentation. This disorder was first discovered in 2016 and there are fewer than 30 known cases worldwide.

Case 7

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

Case 8

Case 8 was born full term and presented with episodes of apnea at day 3 of life which was managed with invasive ventilation and attributed to gastroesophageal reflux. He was again readmitted at day 20 of life for apneas requiring non-invasive ventilation. He had a normal airway assessment, impedance study, and electroencephalography. Magnetic resonance imaging of the brain showed concerns for hypomyelination of the frontal lobes, and child was started on phenobarbitone for concerns of seizures as a cause for the apneas. He was also referred to the genetics team for concerns of congenital hypoventilation syndrome and was recruited for RapidSeq which initially returned negative.

The child subsequently remained well with no further episodes of apneas, and was weaned off anti-epileptic medications from 1 year old. He re-presented at 2 years old with disproportionate short stature, and re-analysis of RapidSeq data showed a pathogenic variant in the *FGFR3* gene, consistent with a diagnosis of hypochondroplasia. This variant had been found in the initial analysis but decision had been made not to report it as clinically the child did not have any features of hypochondroplasia, and incidental findings were chosen not to be reported for this research.

Case 9

Case 9 was a preterm infant born at 34+4 weeks with multiple congenital anomalies including tracheo-esophageal fistula, anorectal malformation, left congenital talipes equinovarus, right multicystic dysplastic kidney and low-lying cord. He underwent RapidSeq at day 3 of life which returned negative.

Case 10

Case 10 was born premature at 35+3 weeks of gestation with perinatal depression requiring intubation at birth. He was noted to have dysmorphic features at birth with a short webbed neck, prominent epicanthal folds, and low set dysplastic and posteriorly rotated ears; hepatosplenomegaly and a moderate fenestrated atrial septal defect, moderate patent ductus arteriosus, tethered pulmonary valve and right ventricular hypertrophy; and hyperleukocytosis. A chromosomal microarray and RapidSeq were offered at day 3 of life.

The patient was found to have a known de novo pathogenic mutation in the *SHOC2* gene, which has been associated with individuals with Noonan-like syndrome with loose anagen hair. This enabled the medical team to better manage his condition and monitor for associated complications such as short stature and need for regular growth monitoring. The diagnosis also facilitated genetic counselling for family planning.

Supplementary Table S4. Number of variants found

Patient	Analysis method	All	Filtered	Mendeliome	X-linked	Homozygous	De novo	Compound heterozygous	Clinically significant
Case 1	Trio	31700	2603	532	0	5	29	0	5
Case 2	Proband	9205	884	462	-	-	-	-	2
Case 3	Proband	9031	510	242	-	-	-	-	4
Case 4	Proband	9655	504	504	-	-	-	-	2
Case 5	Trio	47705	2277	447	0	2	11	20	-
Case 6	Proband	9429	600	268	-	-	-	-	3
Case 7	Trio	13205	1095	493	18	6	33	0	2
Case 8	Trio	35454	3206	578	0	5	37	0	2
Case 9	Trio	11805	726	406	13	5	6	0	5
Case 10	Trio	11739	735	425	11	1	31	0	4

Supplementary Table S5. Gene(s) of interest

Patient	Phenotype of interest	Gene(s) of interest
Case 1	Lysosomal storage disorders	*GALNS, GUSB, GBA, GLB1, NPC1, ASAH1, SLC17A5, NEU1, CTSA, GNPTAB, LIPA*
	Glycogen storage disease type IV	*GBE1*
	RASopathies	*A2ML1, BRAF, CBL, HRAS, KRAS, MAP2K1, MAP2K2, NF1, NRAS, PTPN11, RAF1, RASA1, RIT1, RRAS, SHOC2, SOS1, SOS2, SPRED1*
	Congenital disorders of glycosylation	*ALG1, ALG11, ALG12, ALG13, ALG2, ALG3, ALG6, ALG8, AL9, ATP6V0A2, B3GLCT, CHST14, COG1, COG2, COG4, COG5, COG6, COG7, COG8, DHDDS, DOLK, DPAGT1, DPM1, DPM2, DPM3, G6PC3, GFPT1, GMPPA, GMPPB, MAGT1, MAN1B1, MGAT2, MOGS, MPDU1, MPI, NGLY1, PGM1, PGM3, PMM2, RFT1, SEC23B, SLC35A1, SLC35A2, SLC35C1, SRD5A3, SSR4, TMEM165, TRIP11, TUSC3*
Case 2		Nil
Case 3		Nil
Case 4		Nil
Case 5	Short rib polydactyly syndrome	*CEP120, DYNC2H1, DYNC2LI1, IFT43, IFT52, IFT80, IFT81, IFT140, IFT172, KIAA0586, INTU, NEK1, SRTD1, SRTD12, TCTEX1D2, TTC21B, WDR19, WDR34, WDR35, WDR60*
Case 6	Bone marrow failure	*AP3B1, BRCA2, BRIP1, CSF3R, CXCR4, DKC1, ELANE, ERCC4, FANCA, FANCB, FANCC, FANCD2, FANCE, FANCF, FANCG, FANCI, FANCL, FANCM, G6PC3, GATA1, GATA2, GFI1, HAX1, LAMTOR2, LYST, MPL, NHP2, NOP10, PALB2, RAB27A, RAC2, RAD51C, RBM8A, RMRP, RPL11, RPL15, RPL26, RPL35A, RPL5, RPS10, RPS17, RPS19, RPS24, RPS26, RPS7, RTEL1, RUNX1, SBDS, SLC37A4, SLX4, SRP72, TAZ, TERC, TERT, TINF2, USB1, VPS13B, VPS45, WAS, WRAP53*
	Thrombotic thrombocytopenic purpura	*ADAMTS13*
	Adrenal insufficiency	*STAR, HSD17B10, CYP17A1, CYP11B1, NR0B1, AAAS, NNT, GCCD3, MC2R, MRAP, TXNRD2*
Case 7		Nil
Case 8		Nil
Case 9		Nil
Case 10	RASopathies	*A2ML1, ACTB, ACTG1, BRAF, CBL, CDC42, HRAS, KAT6B, KRAS, LZTR1, MAP2K1, MAP2K2, MRAS, NF1, NRAS, PPP1CB, PTPN11, RAF1, RASA1, RASA2, RIT1, RRAS, SHOC2, SOS1, SOS2, SPRED1*

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