# Rapid Targeted Genomics in Critically Ill Newborns

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abstract

**BACKGROUND:** Rapid diagnostic whole-genome sequencing has been explored in critically ill newborns, hoping to improve their clinical care and replace time-consuming and/or invasive diagnostic testing. A previous retrospective study in a research setting showed promising results with diagnoses in 57%, but patients were highly selected for known and likely Mendelian disorders. The aim of our prospective study was to assess the speed and yield of rapid targeted genomic diagnostics for clinical application.

**METHODS:** We included 23 critically ill children younger than 12 months in ICUs over a period of 2 years. A quick diagnosis could not be made after routine clinical evaluation and diagnostics. Targeted analysis of 3426 known disease genes was performed by using whole-genome sequencing data. We measured diagnostic yield, turnaround times, and clinical consequences.

**RESULTS:** A genetic diagnosis was obtained in 7 patients (30%), with a median turnaround time of 12 days (ranging from 5 to 23 days). We identified compound heterozygous mutations in the *EPG5* gene (Vici syndrome), the *RMND1* gene (combined oxidative phosphorylation deficiency-11), and the *EIF2B5* gene (vanishing white matter), and homozygous mutations in the *KLHL41* gene (nemaline myopathy), the *GFER* gene (progressive mitochondrial myopathy), and the *GLB1* gene (GM1-gangliosidosis). In addition, a 1p36.33p36.32 microdeletion was detected in a child with cardiomyopathy.

**CONCLUSIONS:** Rapid targeted genomics combined with copy number variant detection adds important value in the neonatal and pediatric intensive care setting. It led to a fast diagnosis in 30% of critically ill children for whom the routine clinical workup was unsuccessful.

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Dr van Diemen conceptualized and designed the study, coordinated the study overall, analyzed and interpreted data, codrafted the initial manuscript, and revised the manuscript; Dr Kerstjens-Frederikse developed the logistics for data collection, collected and interpreted data, codrafted the initial manuscript, and revised the manuscript; Drs de Koning and Bergman designed the study, collected and interpreted data, and critically reviewed the manuscript; Profs Swertz, Sinke, van Ravenswaaij-Arts, and van Langen conceptualized and designed the study, interpreted data, and critically reviewed the manuscript; Prof Wijmenga initiated, conceptualized, and designed the study, interpreted data, and critically reviewed the manuscript; Drs Sikkema-Raddatz and Jongbloed developed laboratory and administrative logistics, interpreted data, and critically reviewed the manuscript; Mrs Meems-Veldhuis developed laboratory, administrative, and analytical logistics, performed laboratory work, and analyzed and interpreted data; Drs Herkert, Rump, and Löhner acquired clinical data, interpreted data, and critically reviewed the

**WHAT'S KNOWN ON THIS SUBJECT:** Clinical decision-making in critically ill newborns is challenging. Whole-genome sequencing offers the possibility to simultaneously test all known disease genes to aid in clinical decision-making but has not been tested in a clinical prospective study.

**WHAT THIS STUDY ADDS:** This prospective study shows that rapid targeted genomics combined with copy number variant detection increases the diagnostic yield in the neonatal and pediatric intensive care setting and has a great impact on clinical decision-making.

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Diagnosing a genetic disease on the basis of clinical presentation in critically ill newborns and small infants can be extremely challenging because the symptoms and features of known genetic syndromes may not be present at birth, may change rapidly, or be difficult to observe in a small child on life support. Moreover, the standard genetic diagnostic workup of sequential testing of disease genes considered in the preliminary diagnosis is time consuming. Because diseases can progress rapidly, warranting clinical intervention, it is of the utmost importance to diagnose these children as soon as possible to enable timely interventions that reduce morbidity, suffering, and mortality and avoid pointless and expensive intensive care.

To date, there are >4400 genetic diseases with known causes that, collectively, explain the majority of infant mortality, particularly in NICUs and PICUs.[1] Of these 4400 genes, mutations in some 3300 have clinical consequences as reported by the Clinical Genomic Database (CGD).[2] In the Netherlands, regular genetic diagnostics for these children include molecular and cytogenetic testing to identify larger, structural chromosomal variations, such as trisomies and microdeletions, as well as single-gene and gene-panel testing in the case of suspected monogenic diseases. Turnaround times for these diagnostic procedures range from 1 week to more than 1 year, especially if multiple, consecutive tests are needed. However, there is an urgent need to speed up this process in critically ill children. Whole-genome sequencing (WGS) offers this possibility by simultaneously testing for the presence of mutations in all known disease genes and for small, numerical chromosomal variants. Proof-of-concept studies have already shown the usefulness of WGS in diagnosing suspected genetic diseases in the acute setting of the NICU, but these were all based on a retrospective design.[3,4] Willig et al[4] reported a method in which almost all Mendelian disease genes ($n$ = 4300) were tested by rapid WGS (STATseq) within 50 hours. They were able to diagnose 20 of 35 infants (57%) with a median time to provisional diagnosis of 23 days.

Here, we present the results of a prospective pilot study in which we aimed to implement rapid genomic diagnostics by performing WGS combined with filtering on a gene panel of 3426 genes in a clinical setting for critically ill newborns and infants. We included patients suspected of having a genetic disease but excluded those with a clear clinical diagnosis for which a single targeted test or gene panel was available. We aimed to provide a genetic diagnosis within 2 weeks.

## METHODS

### Selection of Patients

We studied 23 critically ill children admitted to the NICU and/or PICU in the University Medical Center Groningen (UMCG) (Groningen, Netherlands) over a 2-year period and performed rapid targeted genomics aimed at reaching a diagnosis (see Fig 1). Critically ill was defined as cardiorespiratory insufficiency needing ventilator support (16 of 23) or organ dysfunction (the brain, heart, lungs, liver, or kidneys), which was predicted to be a high risk for cardiorespiratory insufficiency in the near future (7 of 23). Criteria for inclusion were age <1 year at presentation and the presence of 1 or more congenital anomalies and/or severe neurologic symptoms, such as intractable seizures, suggestive of a genetic cause of the disease. Exclusion criteria were clear indications for a specific syndrome that could be tested by targeted analysis of known genes (such as epidermolysis bullosa, spinal muscular atrophy, cystic fibrosis, etc) or structural variations (such as trisomy 21 or microdeletion 22q11). The decision to include a patient was made by a multidisciplinary working group comprising pediatricians, clinical geneticists, technicians, bioinformaticians, and laboratory specialists. Patients did not have exome sequencing or any positive result from genetic testing before inclusion, but all regular genetic and other investigations were performed in parallel, with results later than those of the rapid genetic diagnostics. See Supplemental Table 3 for all genetic tests performed in regular diagnostics. For all patients, we followed the procedure outlined in Fig 2. Rapid targeted genomics was performed in all patients in accordance with the regulations and ethical guidelines of the UMCG (UMCG Medical Ethics Committee approval number 2014092).

### Primary End Points

We measured diagnostic yield, turnaround times, and clinical consequences of a rapid genetic-diagnostic approach using WGS. The turnaround time included the moment of inclusion of the patient in the study, DNA isolation, data generation, data analysis, and data interpretation until provisional genetic diagnosis.

### Counseling and Consent

Parents of patients were counseled by the clinical geneticist before and after the rapid targeted-genomics test. Informed consent covered reporting on the diagnostic results for some 3300 known disease genes based on the CGD[2] and included the option to use full genome sequencing data for analysis after the window of rapid targeted diagnostics. The consent also stated the possibility of detecting incidental findings that would be communicated to the family, although we minimized

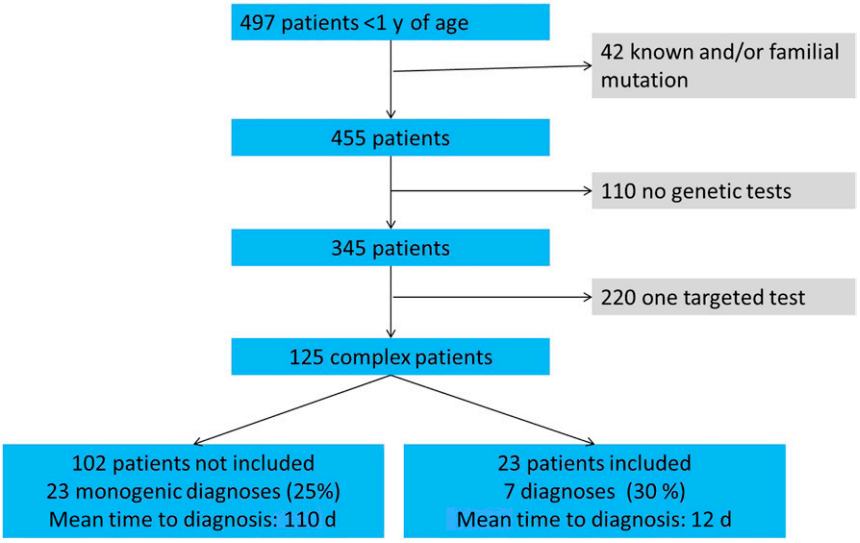


**FIGURE 1**

Overview of inclusion criteria for patients, diagnostic yield, and time to diagnosis. Patients without an obvious diagnosis often need sequential, time-consuming genetic tests. Critically ill children were included in a pilot study to see if rapid WGS led to a diagnosis.

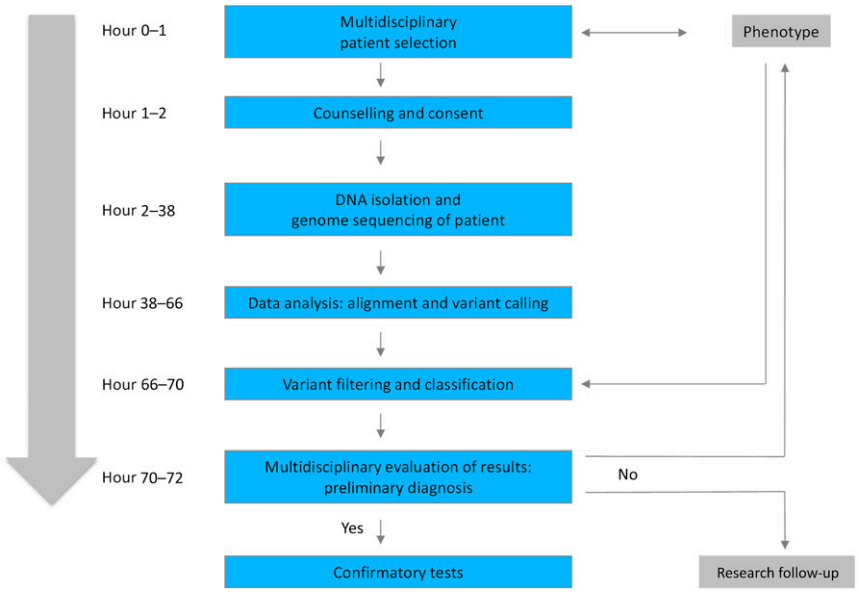


**FIGURE 2**

Schematic overview of the study set-up and time required for each part of the procedure.

the detection of such findings by excluding late-onset disease genes. An independent review board was set up comprising a patient organization representative, a health care lawyer, and a medical ethics specialist to discuss incidental findings, which were predefined as being classified as likely pathogenic or pathogenic mutations in known disease genes not attributing to the patient's current phenotype with preventive options for the health of the patient and/or family (carrier status for autosomal recessive diseases was not reported). When potential actionability was not obvious, the review board was consulted to assist weighing the arguments for and against disclosure.

## DNA Isolation and Sequencing

Blood samples from the patient and both parents were collected for DNA isolation. At the start of the study, we chose WGS instead of whole-exome sequencing to avoid time-consuming capturing steps. DNA from the patient was prepared for WGS according to the procedure described in the Supplemental Information. Because a WGS trio analysis was too expensive, the parents' DNA samples were only used for confirmatory Sanger sequencing of candidate variants and segregation analysis. For quality assurance, 80% of the CGD-based gene panel should be covered at least 20 times, otherwise, additional sequencing data were produced.

## Data Analysis

Raw WGS data from the patients were processed according to standardized protocols as described in the Supplemental Information.[5–8] Sequence variants were filtered by using Cartagenia Next-Generation Sequencing–Bench Laboratory software (Agilent, Santa Clara, CA) by using an automated filtering tree. We generated a virtual gene panel of monogenic diseases based on 3426 genes from the CGD and removed the genes associated with late-onset diseases.[2,9] We further supplemented the gene panel with genes that were on a standard, clinical exome-capturing panel (SureSelect Inherited Diseases; Agilent, Santa Clara, CA) that was used in our genome diagnostics laboratory but not included in the CGD. A full gene list can be found in Supplemental Table 4. We refer to this gene panel as the CGD-based gene panel in the remaining text. Only genes included in the CGD-based gene panel were assessed.

We analyzed variants in the CGD-based gene panel using Human Phenotype Ontology (HPO) terms and minor allele frequencies from 5 databases (1000 Genomes, Genome of the Netherlands, Exome

Sequencing Project 6500, Exome Aggregation Consortium, and the database of single nucleotide polymorphisms) for filtering and performed subsequent annotation with Online Mendelian Inheritance in Man terms, Combined Annotation Dependent Depletion scores, and reported modes of inheritance using MOLGENIS.[10–19] The variants remaining after these filtering steps were manually evaluated for matching with the patients' phenotypes in a multidisciplinary meeting comprising at least the operating technician, a clinical geneticist, and a laboratory specialist. In this way, an average of 40 genes per patient were evaluated. The remaining variants and genes were then classified according to standardized guidelines based on Richards et al[20] by using Alamut software, taking into account (among others) the effect of the candidate variants on the protein as predicted by scale invariant feature transform (SIFT), Polymorphism Phenotyping (PolyPhen), Grantham score, MutationTaster, Align GVGD, and PhyloP. The resulting candidate genes were further evaluated in a larger multidisciplinary working group comprising pediatricians, clinical geneticists, technicians, bioinformaticians, and laboratory specialists. All candidate causal variants and any potential unsolicited findings were validated by using Sanger sequencing in the patient and parents. A detailed description of the variant filtering can be found in the Supplemental Information.

## RESULTS

We included children who were all referred to the UMCG in the Netherlands. Over the time span of this study, the NICU admitted 932 new patients, the PICU admitted 322 new patients <1 year old, and clinical geneticists were consulted for 497 children <1 year old (155 of which were in the NICU or PICU). From 125 complex patients, we considered 30 infants for inclusion in the study. We excluded the following 6 children after discussion: 3 because they were not critically ill (2 children with multiple congenital anomalies and 1 with neonatal cholestasis) and 3 more because we did not have a strong suspicion they had a monogenic disease (1 child with hydrops, 1 with a congenital heart defect and unexplained respiratory failure, and 1 with an omphalocele). Over a period of 2 years (May 2014–May 2016), 24 children met our criteria, but in 1 case, the parents did not give consent for the rapid targeted-genomics test. This led to the inclusion of 23 children in the study (see Fig 1). For 22 of the patients, we also obtained DNA from both parents; for 1 patient, only the mother was available. The median age at inclusion was 28 days (range of 1 day–11 months). Four children presented with cardiomyopathy, 5 with severe seizure disorders, 6 with an abnormal muscle tone, 2 with microcephaly without seizures, 3 with liver failure, 1 with coma because of leukoencephalopathy, 1 with multiple congenital anomalies, and 1 with interstitial pulmonary disease. Table 1 shows the clinical presentations and standardized phenotypes using the HPO terms of the 23 patients included in this study.[21]

For 2 patients, we did not reach the target coverage criteria of 80% of the CGD-based gene panel covered at least 20 times, and we therefore needed to generate additional sequencing data, which delayed the turnaround time by 2 days (1 extra run on the sequencer). Our median turnaround time was 12 days, with a minimum of 5 days and a maximum of 23.

In summary, we identified a causal mutation in 7 of 23 patients, and we had 1 case of an incidental finding (see Table 2 for an overview of the results). Six out of 7 diagnoses were made within the CGD-based gene panel; we identified compound heterozygous mutations in the ectopic P-granules autophagy protein 5 homolog gene (*EPG5*) (Vici syndrome, presenting with microcephaly, seizures, and developmental delay), the required for meiotic nuclear division 1 homolog gene (*RMND1*) (combined oxidative phosphorylation deficiency-11, presenting with microcephaly, seizures, and deafness), the eukaryotic translation initiation factor 2B subunit ε gene (*EIF2B5*) (vanishing white matter, presenting with acute respiratory insufficiency and leukoencephalopathy), the homozygous mutations in the kelch-like family member 41 gene (*KLHL41*) (nemaline myopathy, presenting with severe neonatal contractures), the growth factor ERV1-like gene (*GFER*) (progressive mitochondrial myopathy, presenting with neonatal respiratory distress and lactic acidosis), and the galactosidase β gene (*GLB1*) (GM1 gangliosidosis presenting with cardiomyopathy).[22–29] Lastly, a 1p36.33p36.32 microdeletion was detected in a child with cardiomyopathy by using copy number variant calling on the WGS data.[30,31] The 7 cases are discussed in detail in the Supplemental Information.

### Effects of Rapid Genome Diagnostics on Patient Management

Rapid targeted genomics had a major impact on the decision-making in our clinical services, NICU and PICU, and it led to the withdrawal of unsuccessful intensive care treatment in 5 of the 7 children diagnosed in this prospective group. In addition, concrete diagnoses and appropriate genetic counseling had an impact on the choices parents made for future offspring. Two sets of parents who had decided not to have any more children changed their minds after

**TABLE 1** Overview of 23 Patients' Characteristics in a Prospective Study of Critically Ill Children

| ID | M or F | Age at Presentation | Specific Critical Illness Criteria | Clinical Features | HPO Terms | HPO Codes |
|---|---|---|---|---|---|---|
| 1401 | F | 2 mo | Cardiorespiratory insufficiency, ventilator dependent for 7 d | Lack of spontaneous movements, tremors, no swallowing | Abnormality of the nervous system, abnormality of movement | HP:0000707, HP:0100022 |
| 1402 | F | 4 mo | Cardiorespiratory insufficiency, ventilator dependent for 14 d | Dilated cardiomyopathy | Dilated cardiomyopathy | HP:0001644 |
| 1405 | F | 1 d | Cardiorespiratory insufficiency, ventilator dependent for 11 d | Intractable seizures, perinatal asphyxia | Epileptic encephalopathy, myoclonus, hypotonia | HP:0200134, HP:0001336, HP:0001290 |
| 1406 | M | 10 d | Cardiorespiratory insufficiency, ventilator dependent for 12 d | Intractable seizures, intracerebral hemorrhage | Status epilepticus, myoclonus | HP:0002133, HP:0001336 |
| 1407 | M | 11 mo | West syndrome, inability to swallow | Microcephaly, vermis hypoplasia, West syndrome, developmental delay, VSD | Abnormality of the nervous system, microcephaly | HP:0000707, HP:0000252 |
| 1408 | M | 7 mo | Cardiorespiratory insufficiency, ventilator dependent for 7 d | Prematurity ($24^{+6}$ w), IVF pregnancy, cholestasis | Acute liver failure | HP:0006554 |
| 1501 | F | 5 wk | Hypoventilation, high flow nasal cannula for 8 d | Hydrocephaly, intraventricular hemorrhage | Hydrocephaly, hypotonia, macrocephaly | HP:0000238, HP:0001290, HP:0000256 |
| 1502 | F | 2 wk | Cardiorespiratory insufficiency, ventilator dependent for 10 d | IUGR, seizures, cardiomyopathy, atrioventricular block, PDA, VSD | Cardiomyopathy | HP:0001638 |
| 1504 | F | 9 d | Multifocal seizures, encephalopathic EEG, imminent cardiorespiratory insufficiency | Intractable seizures, dystonia | Abnormality of the nervous system, abnormality of movement | HP:0000707, HP:0100022 |
| 1505 | M | 10 d | Cardiorespiratory insufficiency, ventilator dependent for 8 d | Prematurity ($31^{+2}$ w), coarctation of the aorta, VSD, sagittal craniosynostosis, bilateral postaxial polydactyly, cleft palate, eventration of diaphragm, hypospadias | Malformation of the heart and great vessels, abnormality of the upper limb | HP:0030680, HP:0002817 |
| 1506 | M | 1 d | Cardiorespiratory insufficiency, ventilator dependent for 7 d | Bilateral hip dislocation, flexion contractures, supernumerary nipple, cryptorchidism, consanguineous parents | Flexion contracture, myopathy | HP:0001371, HP:0003198 |
| 1508 | F | 5 mo | Severe hyponatremia (116 mmol/L), imminent cardiorespiratory insufficiency | Microcephaly, dystonia, developmental delay, deafness | Microcephaly, dystonia, developmental delay, profound hearing impairment, hypomyelination | HP:0000252, HP:0001332, HP:0001263, HP:0012715, HP:0003429 |
| 1509 | F | 4 mo | Imminent respiratory insufficiency, continuous positive airway pressure for 3 d | Pulmonary interstitial glycogenosis, consanguineous parents | Interstitial pulmonary disease | HP:0006530 |
| 1511 | F | 4 wk | Cardiorespiratory insufficiency, ventilator dependent for >4 wk | Prematurity (30 w), polyhydramnion, hypotonia, mother muscle weakness | Myopathy, fatigable weakness | HP:0003198, HP:0003473 |
| 1513 | F | 2 wk | Severe hypoglycemia, imminent cardiorespiratory insufficiency; low flow for 3 d | Hypotonia, cholestasis and (transient) hyperinsulinism | Hyperinsulinimia, cholestasis | HP:0000842, HP:0001396 |

**TABLE 1** Continued

| ID | M or F | Age at Presentation | Specific Critical Illness Criteria | Clinical Features | HPO Terms | HPO Codes |
|---|---|---|---|---|---|---|
| 1514 | F | 2 mo | Frequent apnea, imminent respiratory insufficiency | Bulbar weakness and/or palsy, abnormal muscle tone, central hypotonia, mixed hearing loss | Bulbar palsy, abnormal muscle tone, central hypotonia, hearing abnormality, decreased plasma carnitine, hypoglycorrhachia | HP:0001283, HP:0003808, HP:0011398, HP:0000364, HP:0003234, HP:0011972 |
| 1515 | M | 2 wk | Cardiorespiratory insufficiency, ventilator dependent for >4 wk | Cardiomyopathy | Cardiomyopathy, hypertrophic cardiomyopathy, ventricular aneurysm | HP:0001638, HP:0001639, HP:0006698 |
| 1519 | F | 5 d | Cardiorespiratory insufficiency, ventilator dependent for 25 d | Persistent lactic acidosis, dysmorphic features | Abnormality of metabolism and/or homeostasis, abnormal respiratory system morphology | HP:0001939, HP:0012252 |
| 1601 | M | 9 mo | Cardiorespiratory insufficiency, ventilator dependent for 9 d | Sudden seizures | Leukoencephalopathy, abnormal CNS myelination | HP:0002352, HP:0011400 |
| 1602 | M | 9 wk | Cardiorespiratory insufficiency, ventilator dependent for >4 wk | Growth delay, cholestasis, VSDs | Growth delay, cholestasis, ventricular septal defects | HP:0001510, HP:0001396, HP:0001629 |
| 1603 | M | 4 d | Cardiorespiratory insufficiency, ventilator dependent for 13 d | IUGR, microcephaly, epilepsy, encephalopathy, contractures | IUGR, seizures, microcephaly | HP:0001511, HP:0001250, HP:0000252 |
| 1604 | M | 2 mo | Cardiorespiratory insufficiency, ventilator dependent for >4 wk | Abnormal muscle tone, feeding problems | Abnormal muscle tone, muscle weakness, feeding problems | HP:0003808, HP:0001324, HP:0011968 |
| 1605 | F | 3 mo | Cardiorespiratory insufficiency, ventilator dependent for 8 d | Cardiomyopathy, hepatomegaly, consanguineous parents (first cousins) | Cardiomyopathy, hepatomegaly | HP:0001638, HP:0002240 |

CNS, central nervous system; F, female sex; ID, identification number; IUGR, intrauterine growth retardation; IVF, in vitro fertilization; M, male sex; PDA, persistent ductus arteriosus; VSD, ventricular septal defect.

they learned that prenatal testing was available. In the follow-up period (ranging from ~2 years–3 months), no prenatal tests have been performed yet, but presymptomatic testing was performed for 1 couple. In addition, preimplantation diagnostics was chosen by 1 couple. Expanded preconception screening by using a panel restricted to rare serious diseases was offered to all the consanguineous couples after our regular procedure in clinical genetics and was accepted by 1 couple.[31] No additional risk for these diseases was observed in this couple.

### Incidental Findings

The parents of patients were counseled about the chance of incidental findings, although we minimized the detection of such findings by excluding late-onset disease genes. This possibility was clearly stated on the informed consent form. One couple (out of 24) did not agree to the informed consent. For 1 child, we had a suspected case of nonpaternity, which had to be discussed with the parents because the child's diagnosis could not otherwise be confirmed. We had set up an independent review board for incidental findings comprising a patient organization representative, a health care lawyer, and a medical ethics specialist, but there was no need to consult it.

## DISCUSSION

Rapid targeted genomics using WGS has proved to be feasible in our multidisciplinary setting of NICU, PICU, and clinical genetics department in a university hospital in the Netherlands. More importantly, this testing has shown it yields major added value in a routine diagnostics setting with respect to an increased diagnostic yield, in-patient management, and future family planning. In our cohort of 23 critically ill infants, we made 7 diagnoses in patients with a wide range of clinical presentations. All but 1 of these genetic diagnoses would not have been made in our regular molecular diagnostics setting because the diseases were not suspected on clinical grounds and specific genetic testing would not have been considered. The mean turnaround time of 12 days for our rapid targeted-genomic diagnostics suggests that invasive diagnostic testing, such as muscle biopsies, can be avoided in these children in the future. The clinical relevance of rapid genome diagnostics further lies in the fact that these results can be used in the clinical decisions made in caring for critically ill children in ICUs, in better genetic counseling of the parents, and in guiding their future

**TABLE 2** Results of Rapid Genetic Diagnostics of 23 Critically Ill Children

| ID | M or F | Age at Presentation | Clinical Features | Turnaround Time, d | Mean Coverage of CGD-Based Gene Panel | Provisional Diagnosis | Inheritance Mode of Gene | Causal Gene and/or Variants[a] | Allele Frequency ExAC[10] | Reference |
|---|---|---|---|---|---|---|---|---|---|---|
| 1401 | F | 2 mo | Lack of spontaneous movements, tremors, no swallowing | 13 | 51× | No | — | — | — | — |
| 1402 | F | 4 mo | Dilated cardiomyopathy | 14 | 29× | No | — | — | — | — |
| 1405 | F | 1 d | Intractable seizures, perinatal asphyxia | 8 | 33× | No | — | — | — | — |
| 1406 | M | 10 d | Intractable seizures, intracerebral hemorrhage | 13 | 36× | No | — | — | — | — |
| 1407 | M | 11 mo | Microcephaly, vermis hypoplasia, West syndrome, developmental delay, VSD | 23 | 38× | Vici syndrome | Autosomal recessive, compound heterozygous | *EPG5* c.7475T>G (p.Phe2492Cys) and c.5869+1G>A | Not reported before; 0.000008287 | 22 |
| 1408 | M | 7 mo | Prematurity ($24^{+6}$ w), IVF pregnancy, cholestasis | 11 | 30× | No | — | — | — | — |
| 1501 | F | 5 wk | Hydrocephaly, intraventricular hemorrhage | 16 | 32× | No | — | — | — | — |
| 1502 | F | 2 wk | IUGR, seizures, cardiomyopathy, AV-block, PDA, VSD | 13 | 39× | 1p36 microdeletion syndrome[b] | — | — | — | 31 |
| 1504 | F | 9 d | Intractable seizures, dystonia | 20 | 40× | No | — | — | — | — |
| 1505 | M | 10 d | Prematurity ($31^{+2}$ w), coarctation of the aorta, VSD, sagittal craniosynostosis, bilateral postaxial polydactyly, cleft palate, eventration of diaphragm, hypospadias | 15 | 34× | No | — | — | — | — |
| 1506 | M | 1 d | Bilateral hip dislocation, flexion contractures, supernumerary nipple, cryptorchidism, consanguineous parents | 13 | 25× | Nemaline myopathy-9 | Autosomal recessive, homozygous | *KLHL41* c.1213G>A p.(Gly405Ser) | Not reported before | 23 |
| 1508 | F | 5 mo | Microcephaly, dystonia, developmental delay, deafness | 10 | 45× | Combined oxidative phosphorylation deficiency-11 | Autosomal recessive, compound heterozygous | *RMND1* c.1262_1275del (p.Asn421Thrfs*18) and c.713A>G (p.Asn238Ser) | Not reported before; 0.0001741 | 24 |
| 1509 | F | 4 mo | Pulmonary interstitial glycogenosis, consanguineous parents | 13 | 38× | No | — | — | — | — |

**TABLE 2** Continued

| ID | M or F | Age at Presentation | Clinical Features | Turnaround Time, d | Mean Coverage of CGD-Based Gene Panel | Provisional Diagnosis | Inheritance Mode of Gene | Causal Gene and/or Variants[a] | Allele Frequency ExAC[10] | Reference |
|---|---|---|---|---|---|---|---|---|---|---|
| 1511 | F | 4 wk | Prematurity (30 w), polyhydramnion, hypotonia, mother muscle weakness | 17 | 27× | No | — | — | — | — |
| 1513 | F | 2 wk | Hypotonia, cholestasis and (transient) hyperinsulinism | 15 | 43× | No | — | — | — | — |
| 1514 | F | 2 mo | Bulbar weakness and/or palsy, abnormal muscle tone, central hypotonia, mixed hearing loss | 15 | 29× | No | — | — | — | — |
| 1515 | M | 2 wk | Cardiomyopathy | 8 | 37× | No | — | — | — | — |
| 1519 | F | 5 d | Persistent lactic acidosis, dysmorphic features | 7 | 44× | Mitochondrial progressive myopathy | Autosomal recessive, homozygous | *GFER* c.580C>T (p.Arg194Cys) | 0.00001661 | 25·26 |
| 1601 | M | 9 mo | Sudden seizures | 8 | 43× | Leukoencephalopathy with vanishing white matter | Autosomal recessive, compound heterozygous | *EIF2B5* c.1015C>T (p.Arg339Trp) and c.1208C>T (p.Ala403Val) | 0.00004118; 0.000008277 | 27·28 |
| 1602 | M | 9 wk | Growth delay, cholestasis, ventricular septal defects | 8 | 43× | No | — | — | — | — |
| 1603 | M | 4 d | IUGR, microcephaly, epilepsy, encephalopathy, contractures | 7 | 44× | No | — | — | — | — |
| 1604 | M | 2 mo | Abnormal muscle tone, feeding problems | 5 | 45× | No | — | — | — | — |
| 1605 | F | 3 mo | Cardiomyopathy, hepatomegaly; consanguineous parents (first cousins) | 5 | 39× | GM1 gangliosidosis | Autosomal recessive, homozygous | *GLB1* c.176G>A (p.Arg59His) | 0.00004141 | 29 |

AV, atrioventricular; ExAC, Exome Aggregation Consortium; F, female sex; ID, identification number; IUGR, intrauterine growth retardation; IVF, in vitro fertilization; M, male sex; PDA, persistent ductus arteriosus; VSD, ventricular septal defect. —, not applicable.

[a] All diagnoses were confirmed by Sanger sequencing.

[b] Diagnosis was made by routine single nucleotide polymorphism array diagnostics and by using copy number variant calling on WGS data.30

reproductive choices. In this respect, the UMCG is now running a trial of preconception screening to detect autosomal recessive disease genes in couples from the general population who wish to start a family.[32]

During the course of our prospective study, we adjusted and optimized the procedure continuously. Because we performed this study in a multidisciplinary setting of clinicians, technicians, researchers, bioinformaticians, and laboratory specialists, the difficult aspects of the procedure came to light quickly during our weekly discussions, and this led to rapid implementation of improvements that varied from modifications in the logistics to dealing with outdated databases. For the last 7 patients included in the study, we were able to reduce the turnaround time from ∼3 weeks to a maximum of 8 days. A significant impact on our turnaround time arose from the acquisition of 2 new Next-Generation Sequencing machines (Illumina NextSeq500) to replace the Illumina HiSeq2500; these proved to be more stable than our Illumina HiSeq2500, and because we acquired 2 machines, we had sequencing capacity readily available because of the redundancy. Finally, one of the most crucial determinants of the diagnostic yield proved to be the choice of HPO terms used for filtering. For example, we initially missed the diagnosis for patient 1506 with nemaline deficiency because we used the HPO term ''myopathy,'' which does not include the *KLHL41* gene for nemaline deficiency. When we relaxed the HPO term to the broader term ''abnormality of the musculature,'' the mutations in *KLHL41* were readily identified. It further proved important to closely monitor and follow-up the phenotype of patients. Initially, we had no diagnosis for patient 1508, but once it became evident that she had also developed hearing disabilities, we reran the analysis including this phenotypic feature and were able to diagnose her condition as combined oxidative phosphorylation deficiency because of mutations in *RMND1.*

Rapid genetic diagnostics for newborns and infants is not yet common practice and has so far mainly been reported by Kingsmore's group.[3,4] The median turnaround time of our study was comparable to that of Kingsmore's set-up, but our diagnostic yield was lower (30% vs 57%). We think this difference can mainly be explained by our strict inclusion criteria (we investigated only those patients who had no clear syndrome diagnosis), and we consider this as a strength of our study. For example, we excluded patients referred to us with suspected coloboma, congenital heart disease, choanal atresia, mental and growth retardation, genital anomalies and ear malformations and hearing loss otherwise known as CHARGE syndrome and epidermolysis bullosa because they could be diagnosed using straightforward, regular diagnostic testing. Our results and the number of diagnoses we achieved illustrate the power of using WGS diagnostic testing in practice. Other groups have reported on using exome sequencing as a diagnostic tool, and they had comparable yields of detecting mutations in known disease genes (in 25%–30% of their patients).[33–37] However, inclusion criteria and turnaround times were different from our study.

Our diagnoses were considered to be provisional because we decided to confirm them by Sanger sequencing. This typically took 1 week. However, they were all confirmed. In the future, we may relax our mandatory confirmation by Sanger sequencing because of the good reproducibility of high-quality sequencing results. This would improve the turnaround time and offer a faster opportunity for clinical intervention.

To assess the potential of targeted genomics on trios (parents and child), we also conducted a retrospective pilot study to analyze deceased newborns with a suspected genetic disease and their parents using a clinical exome-capturing gene panel (SureSelect Inherited Diseases; Agilent, Santa Clara, CA) and the same bioinformatics analysis approach as used for our rapid targeted-genomics approach. The diagnostic yield was similar, with 3 diagnoses out of 8 patients. There was no need for Sanger sequencing to confirm cases of (compound) recessive disease, and it was easier to pick up de novo mutations (although confirmatory Sanger sequencing remained necessary). This may prove to be an alternative, more cost-effective strategy in the future. Future research should also focus on the cost-effectiveness of genetic diagnostics on the basis of targeted genomics in a prospective study, including all critically ill infants in ICUs. Ideally, such a study should incorporate Next-Generation Sequencing of either exomes or whole genomes on patient–parent trios. Although our current analysis method is based on sequencing only the index patient, new technologic advances will lower the cost of sequencing, and we expect WGS on patient–parent trios to become the standard in the near future.

We are now including more patients in our study and following up on all unsolved patients in a research setting, which includes analyzing their full genomes. We are testing additional copy number variant analyses and a gene-prioritization method based on gene coexpression networks. This has already resulted in the diagnosis of 1 patient (included after the initial cohort described here) with congenital myasthenic syndrome caused by compound heterozygous *RAPSN* mutations (1 deletion of exon 8 not detected by the initial analysis and 1 known pathogenic missense mutation c.264C>A, p.Asn88Lys),

which offered the opportunity for targeted therapeutic intervention. Furthermore, we are evaluating the use of parallel RNA sequencing alongside targeted genomics to help pinpoint candidate genes and mutations by assessing gene differential expression, allele-specific expression, and by analyzing the effect of mutations on splicing.

## CONCLUSIONS

Rapid targeted genomics using WGS has proven to be feasible and fast in our multidisciplinary setting, and the results add major value to the clinical decisions made in the care of critically ill children. Adapting a targeted genomics-first approach enables genetic diagnoses to be reached within a median time of 12 days (range of 5–23 days) in cases that would otherwise require regular, sequential diagnostics lasting 6 months or more. Rapid genome diagnostics raises possibilities to adjust the treatment of critically ill children and perform presymptomatic and prenatal testing.


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## ABBREVIATIONS

CGD: Clinical Genomic Database
EIF2B5: eukaryotic translation initiation factor 2B subunit ε
EPG5: ectopic P-granules autophagy protein 5 homolog
GFER: growth factor ERV1-like
GLB1: galactosidase β 1
HPO: Human Phenotype Ontology
KLHL41: kelch-like family member 41
RMND1: required for meiotic nuclear division 1 homolog
UMCG: University Medical Center Groningen
WGS: whole-genome sequencing

manuscript; Drs Neerincx and van der Velde developed computational pipelines for data analysis, interpreted data, and critically reviewed the manuscript; and all authors approved the final manuscript as submitted.


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