ESHG

BRIEF COMMUNICATION

# Rapid exome sequencing in critically ill infants: implementation in routine care from French regional hospital's perspective

Constance F. Wells[1], Guilaine Boursier[1], Kevin Yauy[1,2,3], Nathalie Ruiz-Pallares[1], Déborah Mechin[1], Valentin Ruault[1], Mylène Tharreau[1], Patricia Blanchet[1], Lucile Pinson[1], Christine Coubes[1], Marc Fila[4], Julien Baleine[5], Odile Pidoux[5], Maliha Badr[5], Christophe Milesi[5], Gilles Cambonie[5], Renaud Mesnage[5], Maëlle Dereure[6], Olivier Ardouin[7], Thomas Guignard[8], David Geneviève[1], Mouna Barat-Houari[1] and Marjolaine Willems[1,9,10]✉



This monocentric study included fifteen children under a year old in intensive care with suspected monogenic conditions for rapid trio exome sequencing (rES) between April 2019 and April 2021. The primary outcome was the time from blood sampling to rapid exome sequencing report to parents. All results were available within 16 days and were reported to parents in or under 16 days in 13 of the 15 individuals (86%). Six individuals (40%) received a diagnosis with rES, two had a genetic condition not diagnosed by rES. Eight individuals had their care impacted by their rES results, four were discharged or died before the results. This small-scale study shows that rES can be implemented in a regional University hospital with rapid impactful diagnosis to improve care in critically ill infants.

*European Journal of Human Genetics* (2022) 30:1076–1082; https://doi.org/10.1038/s41431-022-01133-7

## INTRODUCTION

In severely ill children hospitalized in the neonatal intensive care unit (NICU) or pediatric intensive care unit (PICU), a quick etiological diagnosis is key to proper care management. The phenotypic and genetic variability of severe early genetic conditions and the lack of developmental data make a phenotype-first approach challenging. Exome (ES) and genome sequencing (GS) have been used in research studies for decision-making and care of critically ill patients with a proof-of-principal in 2012 [1] then in larger studies when the turn-around time from blood sampling to results became compatible with the time constraints of the NICU/PICU [2–6].

In France, current genetic testing with no phenotypic diagnosis includes as first-tier testing array-comparative genomic hybridization array (CGH-array). Further testing is carried out using gene panels, ES or GS. This lengthy process does not suit the timeline of critical care. The aim of this study was to evaluate the possibility of on-site rES in a French regional university hospital of getting a rapid genetic diagnosis in critically ill infants.

## METHODS

### Patients

The inclusion criteria were: children under one year admitted to intensive care who would benefit from an early diagnosis as evaluated by the NCIU/PCIU team, suspicion by a consultant of the clinical genetics team of a monogenic condition (i.e.,: multi-systemic involvement, severity of symptoms, consanguinity), both biological parents available (Fig. 1). Patients were excluded if a genetic diagnosis was clinically recognizable, if a chromosomal anomaly was thought more likely or if another non-genetic condition could explain the phenotype.

### Study design and ethics

This prospective single-center study recruited patients from April 2019 to April 2021 who were admitted in the NICU or PICU of the University hospital of Montpellier. Informed signed consent was obtained from both parents. This study was accepted by the ethical committee of Montpellier university hospital (CNRIPH 18.07.17.33927).

### Outcomes

The primary outcome was the number of results reported to parents within 16 days from blood sampling. Secondary outcomes were the number of diagnosis, the number of cases where rES led to a change in medical care, as defined by a treatment, test, action performed because of the rES results.

### Clinical data and concomitant genetic testing

Clinical data was collected from medical records and phenotype was described using the human phenotype ontology (HPO) [7] terms and detailed in Table 1. Patients were clinically reevaluated at 3 months and a year after the inclusion. Other genetic tests, including array-CGH if indicated, were performed as part of standard genetic testing.

[1]Department of Medical Genetics, Rare diseases and Personalized medicine, CHU Montpellier, Univ Montpellier, Montpellier, France. [2]Institute of Advanced Biosciences, Centre de recherche UGA, Inserm U 1209, CNRS UMR 5309 Grenoble, France. [3]SeqOne Genomics, Montpellier, France. [4]Pediatric Nephrology department, Montpellier university hospital, Univ, Montpellier, France. [5]Department of Neonatal Medicine and Pediatric Intensive Care, Montpellier university Hospital, Univ, Montpellier, France. [6]Clinical research and epidemiology department, Montpellier university hospital, Univ, Montpellier, France. [7]Molecular medicine and genomics platform, Montpellier university hospital, Montpellier, France. [8]Unit of Chromosomal Genetics and Research Plateform Chromostem, Montpellier university hospital, Univ, Montpellier, France. [9]Inserm U1298, INM, CHU Montpellier, Univ. Montpellier, Montpellier, France. [10]Reference Centre AD SOOR, AnDDI-RARE, Competence Centre for Rare Skeletal Disorders, OSCAR Network, Montpellier, France.
✉email: m-willems@chu-montpellier.fr

Received: 4 April 2022 Revised: 18 May 2022 Accepted: 9 June 2022
Published online: 22 June 2022

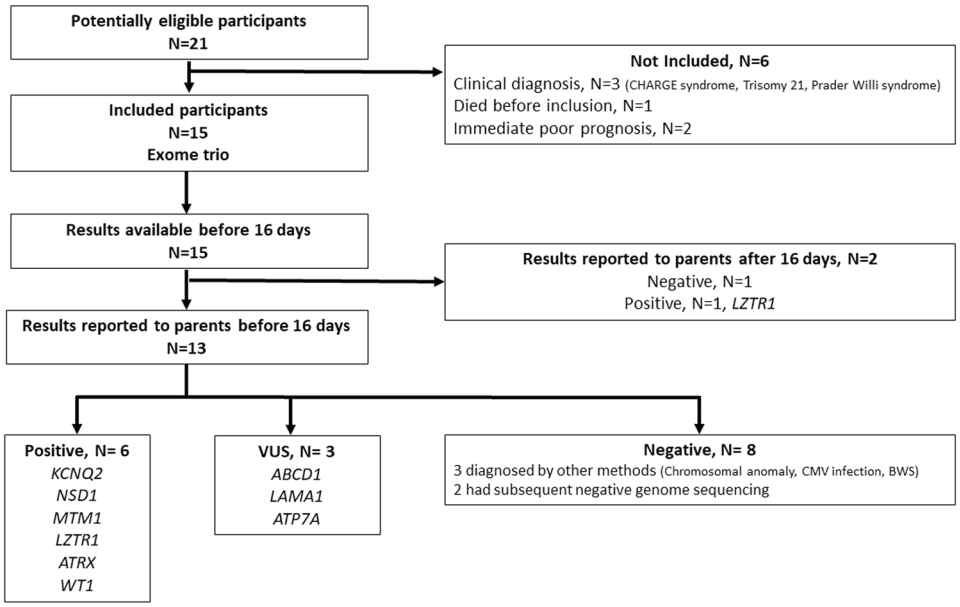


**Fig. 1 Flowchart of included individuals.** BWS Beckwith Wiedemann syndrome, CMV Cytomegalovirus.

### ES processing and interpretation

DNA was extracted from a venous blood sample. We used the SureSelect QTX Library prep (Agilent technologies) enrichment kit and SureSelect Human All Exons V7 (Agilent technologies) following the manufacturer's instruction. Sequencing was performed on a NextSeq 500 (Illumina). Bioinformatics were performed following Broad institute GATK best practice guidelines [8]. Pipeline for variant calling and prioritization is reported in literature [9].

rES data was jointly interpreted by molecular biologists and clinical geneticists in a multi-disciplinary approach. Classification of variants followed the recommendations of the American College of Medical Genetics and Genomics (ACMG) [10]. The report was written by the molecular biologist. Variants were confirmed with Sanger sequencing and a new report issued.

## RESULTS

### Population

Fifteen patients were included in this study. The median age at blood sampling was 38 days (extremes: 4–272; interquartile range: 8–82). Phenotypes of patients are summarized in Table 1; results and impact on care in Table 2. Prenatal invasive genetic testing was carried out in 5 mothers of included infants.

### Primary outcome

Results were reported to parents within 16 days from blood sampling in 13/15 cases (86%), with a median of 14 days (extremes 9–145; interquartile range: 13–15; Table 2). All rES results were available within 16 days. For individual 4, the parents declined the scheduled genetic consultation, because of their child's death. For individual 7, the parents were not available for a consultation before day 16. Of the 15 children, two died before the results.

### Secondary outcome

*Number of diagnoses after rES analysis.* A diagnosis was made by rES in 6 individuals (40%, Table 2). Concomitant tests are summarized in Table 1. In individual 13 diagnosis was made by array-CGH (concomitant genetic test) who had a diagnosis of complex chromosome 18 rearrangement. A variant of unknown significance (VUS) was found in *LAMA1* (Laminin alpha-1, MIM 150320) is in trans of an interstitial deletion due to the complex chromosomal rearrangement which could partly explain the phenotype [11]. Individual 5 had a diagnosis of Beckwith Wiedemann syndrome with mosaic paternal uniparental disomy. In individual 11, because of the severity of the phenotype, a double diagnosis (genetic and a cytomegalovirus infection) was suspected. rES overturned this hypothesis.

*rES impact in patient's care.* The diagnosis changed patients' care in patients where rES was positive in four individuals. In individual 1 with a pathogenic variant in the potassium voltage-gated channel subfamily Q member 2, (*KCNQ2*, MIM 602235), anti-epileptic drugs were switched to carbamazepine, rapidly stopping the seizures. For individual 2, as rES excluded a severe metabolic disorder, the ventricular septal defect was repaired. Individual 3 who had a myotubularin gene variant (*MTM1*, MIM 300415) had his muscular biopsy cancelled and was evaluated for a clinical trial of gene therapy (Clinical trial "ASPIRO", NCT03199469). In individual 15, the *WT1* variant (WT1 transcription factor, MIM 607102) explained the terminal renal failure. Because of the increased tumor risk, she underwent bilateral gonadectomy and nephrectomy with a diagnosis of nephroblastoma at pathological examination. Caryotype further revealed 46, XY chromosomes.

Negative results were also useful in three individuals. For individual 5, rES eliminated differential diagnoses so active care was continued as Beckwith Wiedemann syndrome generally has a good prognosis when properly managed [12]. For individuals 9 and 10, the negative results helped the pediatric team to offer invasive surgical procedures that would not have been considered had there been a severe genetic disease diagnosed by rES.

In individual 8 a VUS was found in *ATP7A* (ATPase Copper Transporting Alpha, MIM 300011), the gene responsible for Menkes syndrome. After review by the clinical genetics team and the molecular biologists, this variant was reported, because further biochemical testing was necessary with a potential treatment [13].

A VUS was also found in *ABCD1* (ATP Binding Cassette Subfamily D Member 1, MIM 300371, c.896A > G;p.His299Arg) responsible for adrenoleukodystrophy [14] in individual 15. Very long fatty chain screening showed slightly increased C26 at 1.38 µmol/L (normal 0.3–1.2). Familial segregation showed that two maternal great-uncles carry the variant with normal very long fatty acid chain screening re-classifying this variant as likely benign, reassuring for her (male karyotype), and family counselling.

**Table 1.** Phenotype of included individuals.

| | Patient 1 | Patient 2 | Patient 3 | Patient 4 | Patient 5 | Patient 6 | Patient 7 | Patient 8 | Patient 9 | Patient 10 | Patient 11 | Patient 12 | Patient 13 | Patient 14 | Patient 15 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| sex | Female | Female | Male | Male | Female | Male | Male | Male | Male | Female | Male | Male | Male | Female | Female |
| Consan-guinity | No | No | No | Yes, first cousins | No | No | No | No | No | No | No | No | No | No | No |
| Family history | No relevant family history | No relevant family history | Several deaths in neonate boys on the maternal side | unexplained death of maternal uncle at 7 days, great-cousin with develop-mental delays | No relevant family history | No relevant family history | Several unrelated *de novo* genetic disorders | Sudden infant death syndrome in a maternal great-cousin and paternal great cousin | No relevant family history | No relevant family history | No relevant family history | No relevant family history | No relevant family history | Family history of feeding difficulties in the father's family | No relevant family history |
| Pregnancy | Midface retrusion at second trimester ultrasound | High-risk first trimester serum markers (1/15), VSD on 2nd trimester ultrasound, type 2 diabetes | Hhydramnios on this twin with amniotic rupture and premature birth | Normal | Preeclmapsia at 38 GW | Increased nuchal translucency, unilateral hypoplasic femur, malformation of D7 vertebrae, delayed bone maturing, preterm birth threat at 32 GW | Normal | Oligoamnios at the end of pregnancy | Staganation of estimated fetal weight in the last three weeks of pregnancy | Generalized edema on third trimester ultrasound | Intra uterine growth retardation | 3rd trimester: small for gestationnal age with unilateral pyelectasis and small left ventricule, gestationnal diabetes | Gestationnal diabetes and hydramnios | Bichorial biamniotic pregnancy, IUGR and oligoamnios on this twin | Normal |
| Birth | Vaginal birth | Planned C-section | Vaginal birth | Vaginal birth | C-section | Vaginal birth | Planned C-section for maternal reason (history of repreated C-section) | Instrumental vaginal birth | Planned C-section (placental position and breach position) | Vaginal birth | Emergency c-section for cardiac rythm anomalies | Emergency c-section for cardiac rythm anomalies | Planned C-section for transverse prense-ntation | C-section | Vaginal birth |
| Term | 39 and 5 days | 38 and 3 days | 30 and 5 days | 38 | 38 and 6 days | 37 | 38 and 3 days | 41 and 1 day | 39 | 35 and 4 days | 29 and 2 days | 36 and 2 days | 39 and 2 days | 32 and 1 day | 40 and 3 days |
| birth weigth (g) (centiles) | 3770 (90th) | 3985 (98th) | 1165 (21th) | 3490 (77,5th) | 3170 (64th) | 2190 (2nd) | 3910 (95th) | 2630 (0,9th) | 2430 (0,6th) | 3235 (97th) | 780 (1st) | 2210 (7th) | 3185 (35th) | 1570 (39th) | 3550 (69th) |
| birth length (cm) (centiles) | 48 (27th) | 53 (99th) | 38 (26th) | 51,5 (86th) | 48 (43rd) | 42 (0,1th) | 50 (68th) | 50 (32th) | 47 (6th) | 52 (100th) | 31 (0,24th) | 45 (14th) | 54 (99th) | 42 (52th) | 51 (76th) |
| birth HC (cm) (centiles) | 35 (75th) | 38 (100th) | 27 (20th) | 35,5 (80th) | 34 (58th) | 33 (29th) | 34 (42th) | 34 (17th) | 32,5 (5th) | 33,5 (83th) | 22 (0,04th) | 31 (7th) | 35,3 (66th) | 29 (33th) | 34 (37th) |
| APGAR at 1/5/10 minutes | 10/10/10 | 6/9/10 | 5/6/7 | 1/2/6 | 6/8/10 | 10/10/10 | 4/9/10 | 2/5/7 | 9/10/10 | 1/7/9 | 2/8/8 | 5/9/10 | 4/5/5 | 9/9/9 | NA/10/NA |
| Neonatal period | Neonatal seizures | Transitory respiratory distress | Hospitalized at birth | Transitory respiratory distress with a two day hospita-lization | Normal until 4 h of life | Hospitalized at birth, ICU from 15 days old | Transitory respiratory distress | Transitory respiratory distress treated with non-invasive ventilation, at 2 hours of life, hypotonia and abnormal nouvements | Transitory hypothermia, hypocal-cemia, gastro-esophageal reflux | Hospitalized at birth | Hospitalized at birth | Hospitalized at birth | Hospitalized at birth | Hospitalized at birth | Normal |
| Cause for ICU entry | Neonatal seizures | Hypotonia and cardiac failure | Prematurity | Seizures, renal failure and metabolic anomalies | Hypoglycemia | Bradycardia and respiratory distress | Seizures | Hypotonia and abnormal mouvements | Post-operative care | Generalized edema | Prematurity and small for gestationnal age | Respiratory distress | Respiratory distress and hypotonia | Prematurity | End stage renal failure |
| Age at ICU entry | 2 days | 1,5 months | at birth | 6 days | 4 h | 15 days | 9 days | 2 h | 2,7 months | at birth | at birth | at birth | at birth | at birth | 9 months |
| Clinical features (HPO terms) | HP:0200134: Epileptic encephalopathy; HP:0000272: Malar flattening; HP:0000158: Macroglossia; HP:0001319: Neonatal hypotonia | HP:0009062: Infantile axial hypotonia; HP:0002509: Limb hypertonia; HP:0001629: Ventricular septal defect; HP:0001684: Secundum atrial septal | HP:0001622: Premature birth; HP:0009062: Infantile axial hypotonia; HP:0002643: Neonatal respiratory distress; HP:0001643: Patent ductus | HP:0005575: Hemolytic-uremic syndrome; HP:0100952: Enlarged sylvian cistern; HP:0002334: Abnormality of the cerebellar | HP:0000105: Enlarged kidney; HP:0001639: Hypertrophic cardiomy-opathy; HP:0006277: Pancreatic hyperplasia; HP:0009890: High anterior | HP:0010880: Increased nuchal translucency; HP:0005613: Aplasia/ hypoplasia of the femur; HP:0002750: Delayed skeletal maturation; | HP:0001250: Seizures; HP:0000836: Hyperth-yroidism | HP:0008846: Severe intrauterine growth retardation; HP:0008935: Generalized neonatal hypotonia; HP:0003131: Cystinuria; HP:0003297: | HP:0002269: Heterotopias/ abnormal migration; HP:0100631: Neoplasm of the adrenal gland; HP:0001579: Primary hypercor-tisolism; | HP:0010310: chylothorax; HP:0007430: generalized edema | HP:0008846: Severe intrauterine growth retardation | HP:0001319: Neonatal hypotonia; HP:0001631: Atrial septal defect; HP:0100581: Dilatation of renal calicies; HP:0002015: Poor | HP:0006955: Olivopont-ocerebellar hypoplasia; HP:0009879: Simplified gyral pattern; HP:0006956: Dilation of lateral ventricles; HP:0007370: | HP:0004602: Cervical C2/ C3 vertebral fusion; HP:0000902: Rib fusion; HP:0011968: Feeding difficulties; Decreased head circumf- | HP:0000286: Epicanthus; HP:0000083: Renal insufficiency; HP:0011369: Mongolian blue spot; HP:0004734: Renal cortical microcysts; HP:0033132: |

**Table 1.** continued

| | Patient 1 | Patient 2 | Patient 3 | Patient 4 | Patient 5 | Patient 6 | Patient 7 | Patient 8 | Patient 9 | Patient 10 | Patient 11 | Patient 12 | Patient 13 | Patient 14 | Patient 15 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | defect; HP:0001181: Adducted thumb; HP:0001520: Large for gestational age | arteriosus; HP:0001972: Macrocytic anemia; HP:0100807: Long fingers | vermis; HP:0012460: Dysmorphic inferior cerebellar vermis; HP0006955: Olivoponta-ocerebellar hypoplasia; HP0000260: Large anterior fontanel; HP0002119: Enlarged ventricular system | hairline; HP:0000158: Macroglossia; HP:0000470: Short neck; HP:0001528: Hemihy-pertrophy; HP:0001998: Neonatal hypoglycemia | HP:0001511: Intrauterine growth retardation; HP:0008905: Rhizomelia; HP:0001840: Metatarsus adductus; HP:0001172: Abnormal thumb morphology; HP:0000201: Pierre-Robin sequence; HP:0007466: Midfrontal capillary hemangioma; HP:0011968: Feeding difficulties; HP:0001662: Bradycardia; HP:0012416: Hypercapnia | | Hyperlysinuria; HP:0003532: Ornithinuria | HP:0001181: Adducted thumb; HP:0001511: Intra-utero growth restriction; HP:0000252: Microcephaly; HP:0001319: Neonatal hypotonia | | | swallowing; HP:000463: Anteverted nares | Aplasia/Hy-poplasia of the corpus callosum; HP:0001319: Neonatal hypotonia; HP:0012020: Right aortic arch; HP:0011683: Restrictive ventricular septal defect; HP:0001631: Atrial septal defect; HP:0000316: Hypert-elorism; HP:0000294: Low anterior hairline; HP:0002045: Hypothermia; HP:0003128: Lactic acidosis | erence HP:0040195; HP:0002946: Supernu-merary vertebrae; HP:0002937: Hemiver-tebrae; HP:0003316: Butterfly vertebrae; HP:0030024: Pretragal ectopia | Renal cortical hyperech-ogenicity; HP:0012021: Persistent patent ductus venosus; HP:0003774: Stage 5 chronic kidney disease |
| Other genetic tests performed | Concomitant array-CGH | Prenatal caryotype 46, XX; post-natal concomittant array-CGH, Steinert and Prader Willi syndrome testing | Concomitant array-CGH, Prader-Willi and Steinert, spinal muscular atrophy | Concomitant array-CGH | Concomitant array-CGH and methylation of 11p15.5 | Prenatal caryotype and array-CGH | Epilepsy gene panel analysis was sent before exome sequencing, Array-CGH not performed | Concomitant array-CGH | Concomitant array-CGH, *TP53* sequencing | Prenatal array-CGH | Concomitant array-CGH | Prenatal array-CGH with post-natal results | Concomitan array-CGH | Array-CGH prior to rES | Concomitant array-CGH |

*CK* creatinine kinase, *CMV* cytomegalovirus, *NA* not available.

**Table 2.** Results of included individuals.

| | Patient 1 | Patient 2 | Patient 3 | Patient 4 | Patient 5 | Patient 6 | Patient 7 | Patient 8 | Patient 9 | Patient 10 | Patient 11 | Patient 12 | Patient 13 | Patient 14 | Patient 15 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Age at blood sampling (days) | 7 | 57 | 38 | 20 | 51 | 45 | 16 | 4 | 82 | 4 | 84 | 16 | 8 | 114 | 272 | |
| Results after blood sampling (days) | 9 | 14 | 14 | 13 | 13 | 14 | 11 | 15 | 13 | 14 | 13 | 13 | 14 | 13 | 11 | |
| Parental report (days) | 9 | 14 | 14 | 145 | 13 | 14 | 26 | 15 | 13 | 14 | 13 | 13 | 16 | 13 | 11 | |
| Exome results | *KCNQ2* (NM_172107.4) | *NSD1* (NM_022455.5) | *MTM1* (NM_000252.3) | Negative | Negative | Negative | *LZTR1* (NM_006767.4) | Negative *ATP7A* (NM_000052.7) | Negative | Negative | Negative | *ATRX* (NM_-000489.6) | Negative *LAMA1* (NM_-005559.4) | Negative | *WT1* (NM_024426.6) | *ABCD1* |
| Variant | c.740 C > T p. Ser247Leu | c.5566 C > T p. Lys1856* | c.1792del p. (His598-Metext*23) | | | | c.742 G > A p. Gly248Arg | c.542 C > T p. Thr181Ile | | | | c.736 C > T p. (Arg208Cys) | c.2515 C > T p.(Pro839Ser) | | c.1399 C > T p. (Arg467Trp) | c.896 A > G p. (His299Arg) |
| ACMG classification | 5 | 5 | 5 | | | | 5 | 3 | | | | 5 | 3 | | 5 | 3 |
| ACMG criteria | PS2, PM1, PM3, PP3, PP5, BP1 | PVS1, PS2, PM2, PP5 | PVS1, PM2, PP5 | | | | PS3, PM1, PM2, PP3, PP4 | PM2, PP3 | | | | PM1, PM2, PM5, PP2, PP3, PP5 | PP3, BP1 | | PS1, PS2, PM2 | PM2, PP3 |
| Inheritance | *de novo* | *de novo* | X-linked, maternally inherited | | | | Paternally inherited | X-linked, maternally inherited | | | | Maternally inherited | Maternally inherited | | *de novo* | X-linked, maternally inherited |
| Etiology | Early infantile epileptic encephalopathy 7 (OMIM#613720) | Sotos syndrome (OMIM#614753) | X-linked centronuclear myopathy (OMIM# 310400) | Hemolytic and uremic syndrome and lympho-histiosis with no known genetic cause | Bethwick Wiedeman syndrome due to mosaic paternal unidisomy | Unknown syndrome with no genetic known cause | Noonan syndrome (OMIM#-600574) | Neonatal distress due to birth complications | Unknown syndrome with no genetic known cause | Unexplained chylothorax | CMV infection | *ATRX*-related disorder (OMIM#-309580) | *de novo* complex anomaly of chromosome 18 | No known genetic etiology | Denys Drash syndrome (OMIM#-194080) | |
| Change in patient care | Drug switch to carbamazepam and controlled seizures | Surgical repair of VSD and early intervention | Evalutation for inclusion in gene tranfer study, no muscular biopsy | No change (patient died before the results) | Active care was continued | No change | No change, went home before the results (17 days) | Serum copper and cerulplasmin were tested at time of exome results and at 6 and 12 months | Surgery to remove adrenal glands was carried out | Surgery to close thoracic canal was carried out | No change | Early intervention | No change | No change | Bilateral nephrectomy and gonadectomy, caryotype | |
| Outcome | Alive at 2 years and 11 months, epileptic encephalopathy | Alive at 3 years old, mild developmental delays | Died at 115 days (3months 1/2) | Died at 28 days of multi-organ failure | Died at 2 months and 15 days of respiratory failure | Died at 1 month and 29 days | Alive and well at 2 years old | Alive and well at 1 year and 11 months | After the exome results: *ARX* sequencing, Beckwith Wiedmann syndrome test and genome sequencing were performed with no genetic diagnosis. Alive at 2 years and 3 months | Alive and well at 1 year and 10 months | Alive at 1 year and 8 months | Developmental delays at 1 year and 5 months | Died at 12 days of respiratory failure, before the exome and Array-CGH results | Alive at 1 year and 3 months | Alive at 1 year and 8 months | |

The classification of variants follow the recommendation of ACMG [10]. *OMIM* online medelian inheritance in man.

## DISCUSSION

This monocentric study shows the experience of a French regional university hospital, very close to what can be implemented in clinical routine care. rES was usefull in early diagnosis and medical care. In this study, 6 individuals (40%) received a diagnosis with rES. This yield is comparable to larger studies who have found a variable diagnostic yield (21–72% [15]). Some symptoms have remained unexplained: macroglossia in individual 1 and seizures in individual 7. Seizures have occasionally been reported in individuals with cardio-facio-cutaneous syndrome which is a RASopathy [16] but is not reported as a usual feature of Noonan syndrome including in individuals with *LZTR1* pathogenic variants. A negative exome is usefull while discussing the possibility of palliative care: a poor genetic prognosis can direct towards palliative care and negative rES to continue active invasive care. The usefulness of a negative result has also been previously reported [6]. A positive result limits the number of diagnostic invasive tests and cost [17, 18].

Teams considering this strategy as a first-tier genetic test in critically ill children should implement a protocol for dealing with incidental findings of the ACMG list [19], heterozygosity for a genetic disorder with a high rate of heterozygosity in the general population, and VUS. In the case of VUS, further tests can be needed to specify the pathogenicity of the variant. VUS in individuals 8 and 15 highlights the difficulties arising with the uncertainty of the clinical consequences of VUS, the medical and familial stress generated by the subsequent delay of the additional tests. In the diagnostic clinical setting, teams should have a protocol for reporting VUS and incidental findings [19].

Limits to this study include its small size sample. Other studies have reported quicker turnaround time with the shortest of 3.3 days [6]. In their study, 86% (ultra-rES) and 78% (rES) of parents had the result before their child was discharged or died.

The rapid evolution of genetic sequencing technologies as well as the acceptability of costs involved by health services might be hurdles to a quick introduction of the latest sequencing technologies in routine patient care [6]. Concerning funding of rapid pan-genomic sequencing, rES reduces unnecessary, costly, and sometimes invasive procedures as well as the time to diagnosis. It also allows genetic counselling as well as proper care management of the child according to their diagnosed condition [18]. Parental acceptance is good with little adverse reactions [20]. Hence, the medical, psychological, and monetary benefits of rES outweigh the risks.

In conclusion, rES is feasible in critically ill infants in a French regional university hospital with a good diagnostic yield. It can be offered in individuals for whom a genetic condition is suspected where a diagnosis could help in their care. New studies show a possible switch to rGS to diagnose monogenic diseases but also chromosomal abnormalities. Sequencing technology and costs as well as evolution of data storage will make GS more accessible and may soon become the first-tier genetic test in critically ill children with suspected genetic disorders.

## DATA AVAILABILITY

All data generated or analyzed during this study are included in this published article.

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## ACKNOWLEDGEMENTS

We thank all the families for their involvement in the study. We also thank Dr Marie Christine Picot for her help in the methodology and Dr Florence Masson, Dr Floriane Hemery, Dr Odile Plan for their involvement in patient care.

## AUTHOR CONTRIBUTIONS

CFW, KY, GB, MBH and MW participated in drafting the manuscript and correction of the manuscript. GB, MT, MF, GC, TG, were involved in correction of the manuscript. PB, LP, CC, GC, JB, OP, MBH, CM, GC, RM, MD were involved in the curation of data. KY,

OA, GB, NRP, DM, CW, MBH, VR, MT, KY, OA, TG DG and MW participated in the curation and interpretation data. MW, MD, DG were implicated in the grant applications.

## FUNDING

Funding for this study was obtained thanks to internal research credits of the university hospital of Montpellier ("Appel d'offre interne jeune chercheur").

## COMPETING INTERESTS

The authors declare no competing interests.

## ETHICS APPROVAL

This study was accepted by the ethical committee of Montpellier university hospital (CNRIPH 18.07.17.33927). All parents signed a consent to participate to the study for themselves and their child.

## ADDITIONAL INFORMATION

**Correspondence** and requests for materials should be addressed to Marjolaine Willems.