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Corrected: Author correction

Putting genome-wide sequencing in neonates into perspective

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Purpose: Several studies have reported diagnostic yields up to 57% for rapid exome or genome sequencing (rES/GS) as a single test in neonatal intensive care unit (NICU) patients, but the additional yield of rES/GS compared with other available diagnostic options still remains unquantified in this population.

Methods: We retrospectively evaluated all genetic NICU consultations in a 2-year period.

Results: In 132 retrospectively evaluated NICU consultations 27 of 32 diagnoses (84.4%) were made using standard genetic workup. Most diagnoses (65.6%) were made within 16 days. Diagnostic ES yield was 5/29 (17.2%). Genetic diagnoses had a direct effect on clinical management in 90.6% (29/32) of patients.

Conclusions: Our study shows that exome sequencing has a place in NICU diagnostics, but given the associated costs and the high yield of alternative diagnostic strategies, we recommend to first perform clinical genetic consultation.

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INTRODUCTION

Exome sequencing (ES) and genome sequencing (GS) are increasingly being applied in the neonatal setting. It has been suggested that rapid sequencing is particularly relevant in this population, because major clinical decisions could be affected by a genetic diagnosis.[1,2] The first report of rapid genome-wide sequencing (rES/GS) in neonatal intensive care unit (NICU) patients dates from 2015[2] and reported 2–7 day turnaround times, whereas regular diagnostic turnaround times are currently significantly longer (up to several months). This study found a diagnostic yield of 57%, among the highest in sequencing studies, suggesting that rES/GS should be implemented broadly in NICUs worldwide. However, this paper and other papers reporting rES/GS[2–4] limit their data to cases where rES/GS was primarily applied, and also perform rES/GS in patients with a clinical suspicion of a syndrome. This explains why usually recognizable syndromes, such as CHARGE or Noonan syndrome, are among the diagnoses made in rES/GS reports.[1–3] Therefore, it could be argued that the actual added value of ES/GS in general, and rES/GS in particular, compared with a more classical clinical genetic approach, has not been properly

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assessed. Although no one will doubt that ES/GS will lead to a higher diagnostic yield, it is relevant to know the extent of improvement given the limited availability of ES/GS in many countries, the high associated costs, and the fact that most health-care systems have limited financial resources.

Although a randomized controlled trial would be the best method to determine the added value of ES/GS, such studies would require large sample sizes due to the incredible heterogeneity in NICU presentations, and face additional difficulties due to rapid changes in availability of genetic testing as recently illustrated.[1] We therefore decided to perform a retrospective observational study of all clinical genetic NICU consultations in a 2-year period.

MATERIALS AND METHODS

Selection of patients

All patients who received clinical genetic consultation at the neonatal medium or intensive care (NICU) or pediatric intensive care (PICU) in the Leiden University Medical Center (LUMC) between September 2014 and September 2016 and were aged ≤120 days were included.

A waiver of consent was granted by the Institutional Review Board (IRB) of the LUMC.

Genetic investigations

Before or simultaneously with ES a Cytoscan High DensityArray (ThermoFisher) was performed to detect copy-number variation. ES could consist of either trio ES (including parents) or single patient ES, and consisted of Agilent SureSelect v5 capture followed by Sequencing on Illumina platforms (Hiseq2500 or Hiseq4000). Analysis was performed in the LUMC's clinical genetic laboratory using a GATK-based pipeline[5] and in-house developed analysis software (LOVD+).

ES was performed after clinical genetic consultation, consent from the parents, and if one of the following conditions was met:

- Isolated cardiac anomaly (mostly single ES)
- Combination of multiple congenital anomalies, or a congenital anomaly with dysmorphic features, in the absence of a clinical diagnosis
- Delayed development or, e.g., persisting feeding problems at follow-up (FU)

Data collection

Clinical notes were retrospectively evaluated to determine the referral reason for genetic consultation and document the presenting features including congenital malformations in each patient.

Regarding the outcome of each patient, the following categories were defined:

- Genetic diagnosis: all patients in whom the genetic diagnosis is considered to explain the most important features of their phenotype
- Likely syndromic: patients with multiple malformations, or significant developmental delay on FU, but without genetic diagnosis
- Nongenetic: patients with alternative, nongenetic causes that likely explained their complete phenotype
- Isolated/spontaneously resolving: patients with normal development on FU, in whom either the phenotype had disappeared (e.g., hypotonia), or the congenital malformation appeared to be an isolated, most likely multifactorial determined feature
- Lost to FU: patients for whom the appropriate category could not be determined with the available clinical data

To evaluate the effect of a genetic diagnosis on medical management, we used the categories proposed by Meng et al.[3] These categories are redirection of care, initiation of new subspecialist care, changes in medication or diet, and major procedures. To specify the second category further, we made four subcategories: positive screening (i.e., additional screening led to detection of additional anomalies), negative screening (i.e., additional screening led to the exclusion of associated features), future screening, and tumor screening.

We determined the time to diagnosis as the time between the consultation and the clinical or molecular diagnosis, whichever came first. A diagnosis was considered fast when it was made within 16 days after consultation. A clinical diagnosis was recorded when the diagnosis was communicated to the parents as the most likely diagnosis and informed clinical management.

Statistics

All performed statistical tests were two-sided. The applied tests are indicated in the respective tables. No multiplicity correction was performed.

RESULTS

Patient characteristics

One hundred thirty-two infants received a genetic consultation and were retrospectively included within the 2-year observation period (Fig. 1, Table 1). Most of these patients (94.7%, 125/132) were seen at the NICU or PICU, while the others were seen in neonatal medium care settings ($n = 7$). At some time during their admission three of these seven patients were admitted to the NICU. The most frequent referral reasons were congenital cardiac anomalies (39%, 51/132), dysmorphic features (11%, 14/132), and brain anomalies (8%, 11/132). Clinical genetic consultation took place at a median age of 3 days after birth (range 0–115 days). In four patients molecular diagnostics had been performed prenatally, leading to a presumptive diagnosis. The duration of IC admission varied from 0 to 129 days with a median of 10 days. Of the included patients 20 died (age of death 0–655 days, median 14 days); ES was performed in 7 of these 20 patients.

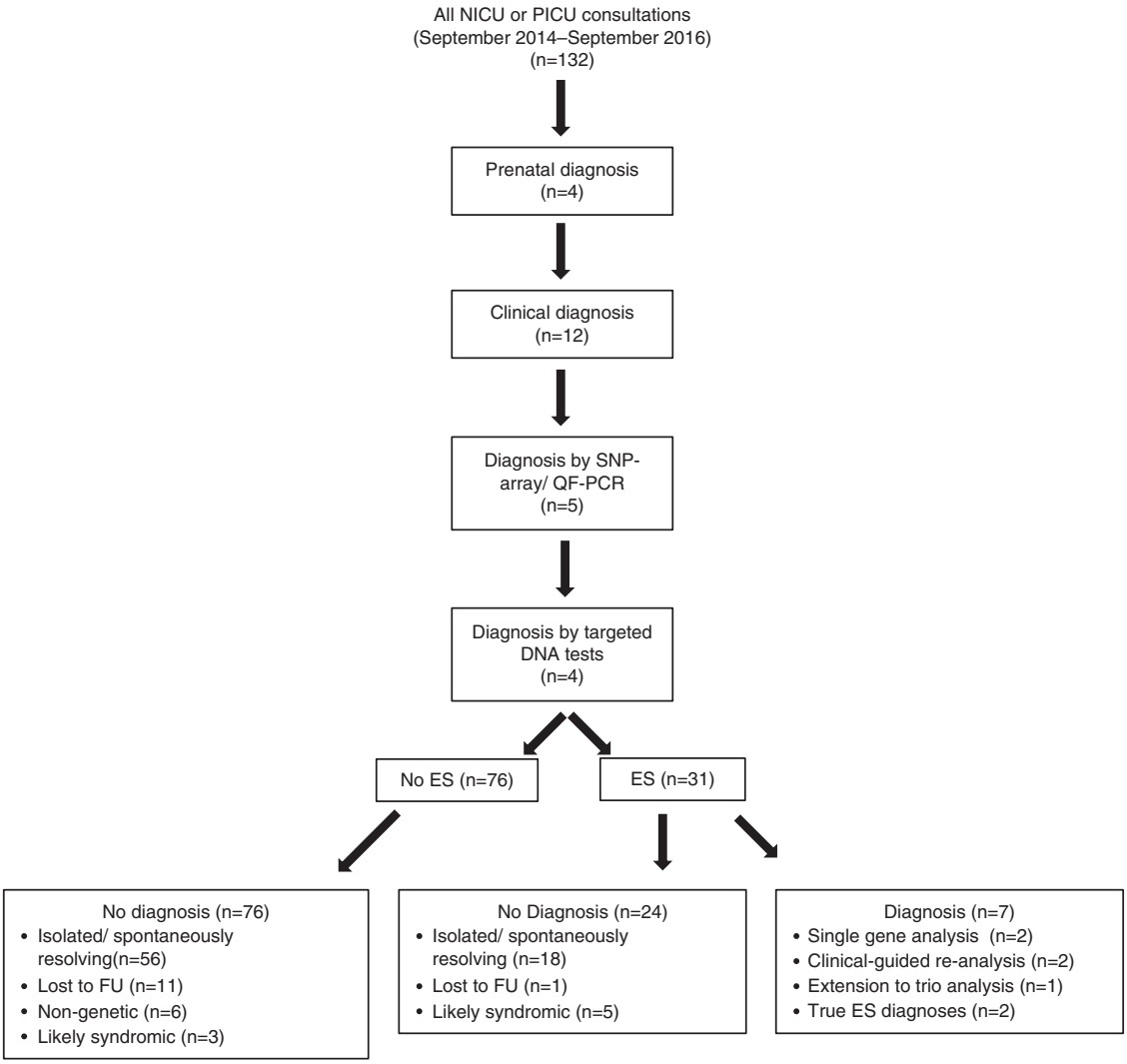

Fig. 1 Schematic overview of study results. *ES* exome sequencing, *FU* follow-up, *NICU* neonatal intensive care unit, *PICU* pediatric intensive care unit, *QF-PCR* quantitative fluorescence polymerase chain reaction, *SNP* single-nucleotide polymorphism.

Diagnostics

In 32/132 patients (24.2%) a genetic diagnosis was obtained (Table 2 and Table S1). Excluding Down syndrome and prenatal diagnoses the diagnostic yield was 18.0% (22/122). A substantial number of these diagnoses were clinical diagnoses based on the pattern of congenital malformations and dysmorphic features (37.5%,12/32, excluding Down syndrome 26.9%, 7/26). All clinical diagnoses were made on the day of consultation, except for the diagnosis of Kabuki syndrome, which was made 11 days later, and all were eventually confirmed by molecular genetic testing (0–69 days, median 11; excluding Down syndrome 5–69 days, median 25). In addition, one suspicion of Down syndrome was confirmed using QF-PCR and eight diagnoses were made using array analyses, including four prenatal diagnoses. Array results were usually obtained within 2 weeks after the genetic consultation (excluding prenatal diagnoses: range 10–17 days, median 14 days), resulting in a total of fast diagnoses of 60.7% (17/28) (excluding prenatal diagnoses: median 0 days, range 0–16 days). Eleven late diagnoses were made 34–991 days (median 164 days) after the first genetic consultation. In four of these cases, a targeted molecular test (such as a Noonan gene panel) with relatively long turnaround time was requested because of a clinical suspicion (55–311 days, median 115 days).

Most of the 100 patients without a genetic diagnosis were classified as isolated/spontaneously resolving ($n = 74$, 74.0%), while 12 were lost to follow-up, 6 were classified as nongenetic, and 8 were categorized as likely syndromic. In 5 of these 8 likely syndromic patients, ES was performed but did not yield a diagnosis.

Exome sequencing

ES was performed in 31/132 patients (24 trio ES, 7 single ES). The time to request ES after consultation ranged from 0 to 499 days with a median of 124 days. In two cases a positive ES result was obtained after a single gene was analyzed from the ES data because of a prior clinical suspicion (*CHRNG*, *CHD7*).

Table 1 Patient characteristics

	All patients ($n = 132$)			Non-ES patients ($n = 101$)			ES patients ($n = 31$)				
	Total	*n*	%	Total	*n*	%	Total	*n*	%	*p* value	Test
Age at consultation (days), (nr, median [min–max])	132	3	(0–115)	101	3	(0–115)	31	3	(0–39)	0.593	[a]
ICU category	132			101			31			0.190	[b]
NICU		105	80%		81	80%		24	77%		
PICU		20	15%		14	14%		6	19%		
MC		7	5%		6	6%		1	3%		
Referral reason	132			101			31			0.343	[b]
Cardiac anomaly		51	39%		34	34%		17	55%		
Dysmorphic features		14	11%		12	12%		2	6%		
Brain anomaly		11	8%		8	8%		3	10%		
MCA		8	6%		6	6%		2	6%		
Suspected Down syndrome		7	5%		7	7%		0	0%		
Hydrothorax		6	5%		6	6%		0	0%		
Convulsions		3	2%		3	3%		0	0%		
Encephalopathy		4	3%		3	3%		1	3%		
Hypotonia/feeding difficulties		4	3%		3	3%		1	3%		
Limb anomaly		3	2%		3	3%		0	0%		
Dysmaturity		3	2%		3	3%		0	0%		
Other (categories $n = 1$)		18	14%		13	13%		5	16%		
Time to genetic diagnosis (days), (nr, median [min–max])	32	220.5	[-174–991]	25	0	[−174–311]	7	512	[34–991]	<0.01	[a]
Outcome	132			101			31			0.037	[b]
Genetic diagnosis		32	24%		25	25%		7	23%		
Likely syndromic		8	6%		3	3%		5	16%		
Nongenetic		6	5%		6	6%		0	0%		
Isolated/spontaneously resolving		74	56%		56	55%		18	58%		
FU		12	9%		11	11%		1	3%		
Deceased	132	20	15%		13	13%		7	23%	0.187	[b]
Effect management of diagnosis[c]	32		32	25			7				
Reproductive counseling		32	100%		25	100%		7	100%	0.527	[b]
Redirection of care		2	6%		2	8%		0	0%		
Initiation of new subspecialist care		20	63%		18	72%		2	29%		
Additional screening positive		2	6%		2	8%		0	0%		
Additional screening negative		11	34%		9	36%		2	29%		
Future screenings		2	6%		2	8%		0	0%		
Changes in medication or diet		1	3%		1	4%		0	0%		
Major procedures		0	0%		0	0%		0	0%		
Diagnosed using	32			25			7			<0.001	[b]
SNP array/QF-PCR		9	28%		8	32%		0	0%		
Targeted genetic testing		4	13%		5	20%		0	0%		
Clinical diagnosis		12	38%		12	48%		0	0%		
Exome sequencing[d]		7	22%		0	0%		7	100%		

ES exome sequencing, *FU* follow-up, *ICU* intensive care, *MC* medium care, *MCA* multiple congenital anomalies, *NICU* neonatal intensive care, *nr* number, *PICU* pediatric intensive care, *QF-PCR* quantitative fluorescence polymerase chain reaction, *SNP* single-nucleotide polymorphism.

[a]Mann–Whitney *U*.

[b]Chi-square.

[c]Per patient more than one consequence was possible.

[d]Two ES diagnoses were made after a single gene was analyzed. Consequently, total ES yield 17.2% (5/29).

Table 2 Characteristics of patients with a genetic diagnosis

Patient ID	Age consultation (days)	Age death (days)	Gene	Inheritance	Disease name	OMIM (disease)	OMIM (gene[s])	Diagnosed using:	Time to diagnosis (days)	Consequences of diagnosis[a]	Future screening	Diagnosis before discharge
1	3	6	*ATAD3A*	Recessive	ATAD3A-related pontocerebellar hypoplasia		*612316; *612317	ES/ reanalysis	991	None (died)[a]		No
2	1		*PTPN11*	Dominant	Noonan syndrome	163950	*176876	Targeted diagnostics	55	Additional screening (negative)	X	No
3	2		*NPC1*	Recessive	Niemann–Pick type C	257220	*607623	Targeted diagnostics	68			No
3	2		*OTOA*	Recessive	Autosomal recessive deafness type 22	607039	*607038	ES-based hearing loss panel	167			No
4	2		*T21*	Chromosomal	Down syndrome	190685	*190685*	Clinical diagnosis	0	Initiation of new subspecialist care	X	Yes
5	1		*T21*	Chromosomal	Down syndrome	190685	*190685*	Clinical diagnosis	0	Initiation of new subspecialist care	X	Yes
6	2		*PTPN11*	Dominant	Noonan syndrome	163950	*176876	Clinical diagnosis	0		X	Yes
7	3		*CDK13*	Dominant	CDK13 syndrome	617360	*603309	ES/ reanalysis	938			No
8	2		Chromosomal	Dominant	22q11 deletion syndrome	187500	*611867*	SNP array	PND	Additional screening (negative); irradiated blood for surgery		Yes
9	4		*SLC6A9*	Recessive	Glycine encephalopathy	617301	*601019	ES/ reanalysis	512			No
10	0		*COL2A1*	Dominant	Spondyloepiphyseal dysplasia congenita	183900	*120140	Targeted diagnostics	311	Additional screening (negative)		No
11	3		*T21*	Chromosomal	Down syndrome	190685	*190685*	QF-PCR	1	Initiation of new subspecialist care	X	Yes
12	0		*T21*	Chromosomal	Down syndrome	190685	*190685*	Clinical diagnosis	0	Initiation of new subspecialist care	X	Yes
13	0		*CHD7*	Dominant	CHARGE syndrome	214800	*608892	ES (targeted readout)	130	Additional screening (negative)	X	No
14	0		*T21*	Chromosomal	Down syndrome	190685	*190685*	Clinical diagnosis	0	Initiation of new subspecialist care	X	Yes
15	3		Chromosomal	Dominant	1q24.3-q31.1del			SNP array	13	Additional screening (negative)		Yes
16	1		Chromosomal	Dominant	1q21.1	612474		SNP array	PND			Yes
17	23		*NSD1*	Dominant	Sotos syndrome	117550	*606681	Targeted diagnostics	162	Additional screening (negative)	X	No
18	4		*PWS*	Dominant (imprinted)	Prader–Willi syndrome	176270		SNP array	15	Early treatment with GH	X	Yes
19	1	655	*CHD7*	Dominant	CHARGE syndrome	214800	*608892	Clinical diagnosis	0	Identification of additional anomalies	X	Yes
20	2	8	Chromosomal	Chromosomal	17q12q25.3dup 12p13.33p13.32dup (high mosaic)			SNP array	PND	Initiation of palliative care		Yes
21	1		*CHRNG*	Recessive	Escobar syndrome	265000	*100730	ES (targeted readout)	130	None[a]		No
22	27		*KMT2A*	Dominant	Wiedemann–Steiner syndrome	605130	*159555	ES	34	Additional screening (negative)	X	No
23	0		*PIK3CA*	Dominant (mosaic)	PIK3CA-related disorders	612918	*171834	Clinical diagnosis	0	Additional screening (negative); tumor screening	X	Yes
24	3	6	*GLDC*	Recessive	Glycine biosynthesis deficiency	605899	*238300	Clinical diagnosis	0	Initiation of palliative care		Yes
25	1		Chromosomal	Chromosomal	46,XX,der(21)t(11;21)(p15.4;q22.2) Beckwith–Wiedemann syndrome	130650		SNP array	10	Tumor screening		Yes
26	1		*CHD7*	Dominant	CHARGE syndrome	214800	*608892	Clinical diagnosis	0	Identification of additional anomalies	X	Yes
27	1		*T21*	Chromosomal	Down syndrome	190685	*190685*	Clinical diagnosis	0	Initiation of new subspecialist care	X	Yes
28	0		*NIPBL*	Dominant	Cornelia de Lange syndrome	122470	*608667	Clinical diagnosis	0	Additional screening (negative)	X	Yes
29	11		Chromosomal	Dominant	18p11.22del	146390		SNP array	16	Additional screening (negative)		No
30	39		*ADNP*	Dominant	Helsmoortel–van der Aa syndrome	615873	*611386	ES	516	Additional screening (negative)		No
31	0	22	*KMT2D*	Dominant	Kabuki syndrome	147920	*602113	Clinical diagnosis	11	None (died)[a]	X (died)	Yes
32	2		Chromosomal	Chromosomal	47,XY,+der(10)t(5;10)(p15.3;q11.2)			SNP array	PND	Additional screening (negative)		Yes

ES exome sequencing, *GH* growth hormone, *MCA* multiple congenital anomalies, *PND* prenatal diagnostics, *QF-PCR* quantitative fluorescence polymerase chain reaction, *SNP* single-nucleotide polymorphism.[a]Parents of all patients received reproductive counseling and prognostic information was available (prognostic information was not given for patients 1 and 31 because they had died at the time of diagnoses, nor for patients 21 and 32 for whom insufficient prognostic information was available).

In two other cases, a diagnosis was initially missed by ES, but clinically guided reanalysis did provide pathogenic variants in newly described genes (*SLC6A9* and *ATAD3A*[6]). In a patient with a heart defect, panel ES did not provide the causative variant, but full trio ES identified a pathogenic variant in *CDK13*. In the remaining 26 ES, 2 diagnoses were made (*KMT2A*, Wiedemann–Steiner syndrome [OMIM 605130] and *ADNP*, Helsmoortel–van der Aa syndrome [OMIM 615873]). The immediate clinical yield was therefore 2/29 (6.8%), while the total yield was 5/29 (17.2%).

Factors influencing diagnostic yield

To assess whether the clinical presentation of patients influenced the probability of obtaining a genetic diagnosis, we investigated the effects of (1) referral reason, (2) influence of the presence or absence of clinical features, and (3) number of affected organ systems.

When observing the odds ratios for obtaining a diagnosis per referral reason (Table 3, left part), it is clear that patients referred because of a suspicion of Down syndrome were most likely to obtain a genetic diagnosis. Patients referred because of encephalopathy and hypotonia/feeding difficulties, as well as patients with limb anomalies also showed high odds ratios, whereas patients referred primarily because of cardiac or brain anomalies showed a relatively low odds ratio. Many of these odds ratios do not differ significantly from 1, which is likely due to the low sample size per referral category. For ES the sample size is even smaller, but it is interesting to note that the diagnostic yield in patients referred primarily because of cardiac anomalies tended to be lower.

Irrespective of the referral reason, we also analyzed whether the presence of anomalies in organ systems influenced the chance of a clinical diagnosis (Table 3). This table shows that all odds ratios are close to and include 1. Only the presence of evident dysmorphic features seems to increase the chance of a diagnosis ($p = 0.074$).

We also evaluated whether the number of affected organ systems influenced the diagnostic yield. No effect was found of the number of reported anomalies on the diagnostic yield (Table 3, bottom).

Consequences of genetic diagnoses

All diagnosed patients received genetic counseling, providing their parents with relevant information for possible future pregnancies. In 90.6% (29/32) of diagnosed patients we were able to inform the parents about the prognosis associated with the genetic diagnosis (Table 2 and Table S1). In the other three patients, either insufficient information was present because limited clinical data were published (*CHRNG*) or the patient had already died (Kabuki syndrome, *ATAD3A*). Disregarding tumor screening, for 16 patients the diagnoses had long-term management consequences such as future screening for disease-associated features. Furthermore, in most patients the diagnosis led to initiation of new subspecialist care (62.5%, 20/32), often additional screening. Screening led to additional findings in two patients (2x CHARGE syndrome [OMIM 214800]). Of the 12 patients where the additional screening showed no abnormalities, 6 had an indication for future screening. In addition, two patients received redirection of care (i.e., initialization of palliative care [$n = 2$]). In two patients the genetic diagnosis led to initiation of a tumor screening program (Beckwith–Wiedemann syndrome [OMIM 130650] and CLOVES syndrome [OMIM 612918]). A change in medication was received by a patient with Prader–Willi syndrome (OMIM 176270); an early treatment with growth hormone was started although there is currently no evidence that such an early start of growth hormone treatment provides additional benefits over a start at 6 months per protocol.

DISCUSSION

Rapid genome-wide sequencing has been advocated as a high yield diagnostic test in NICU patients, but the studies published thus far have not directly assessed the additional yield compared with more classical genetic approaches. In this study, we have retrospectively included all 132 genetic NICU/PICU consultations over a 2-year period. The overall diagnostic yield of ES was 5/29 (17.2%).

Comparison with other NICU studies

Setting

The LUMC functions as an expertise center for neonatal cardiac surgery, whereas other types of neonatal surgery are referred to other hospitals and are lacking from our patient population. Even though we were less strict in the inclusion of patients (medium care patients were also included), and despite our biased patient population, the list of diagnoses seems comparable with diagnoses typically made in other papers.[2–4,7] The presence of multiple patients with Noonan, CHARGE, and Kabuki syndrome and severe early-onset metabolic disorders clearly shows overlap.

Diagnostic yield

Based on our data, the additional yield of ES over other diagnostic tools is 17.2%. We have recalculated the yields of other NICU papers, taking into account unrecognizable or atypical presentations only, and these are relatively similar to our findings (Table 4).

We did not identify particular clinical features that were predictive of the chance of a genetic diagnosis, although the number of included patients per category is small. Meng et al.[3] reported a lower diagnostic rate for patients with cardiac anomalies and a higher rate for patients with "abnormalities of the musculature," and our relatively low odds ratio for a genetic diagnosis in patients referred because of cardiac anomalies seems to corroborate this finding. No association was found between the number of reported features and a genetic diagnosis. This is contrary to the findings of Trujillano et al.,[8] who showed that a higher number of Human Phenotype Ontology (HPO) terms is associated with a higher diagnostic yield. Important differences between these studies are the number of included

Table 3 Relation between referral reason and clinical features on the diagnostic yield for all genetic investigations, and separate for exome sequencing

	All 2-year follow-up patients ($n = 132$)							Exome sequencing patients ($n = 29$)[a]						
	Genetic diagnoses ($n = 32$)		Without genetic diagnosis ($n = 100$)					Genetic diagnoses ($n = 5$)		Without genetic diagnosis ($n = 24$)				
Referral reason[b]	*n*	% per category	*n*	% per category	Odds ratio	*p* value	Test	*n*	% per category	*n*	% per category	Odds ratio	*p* value	Test
Cardiac anomaly	9	18%	42	82%	0.54 (0.23–1.29)	0.161	[c]	1	6%	15	94%	0.15 (0.01–1.56)	0.144	[d]
Dysmorphic features	3	21%	11	79%	0.84 (0.22–3.21)	0.795	[d]	1	50%	1	50%	5.75 (0.30–111.88)	0.320	[d]
Brain anomaly	0	0%	11	100%	-	0.050	[d]	0	0%	3	100%	-	1.000	[d]
MCA	2	25%	6	75%	1.04 (0.20–5.45)	0.959	[d]	1	50%	1	50%	5.75 (0.30–111.88)	0.320	[d]
Suspicion of Down syndrome	6	86%	1	14%	22.85 (2.63–198.22)	<0.001	[c]	0	-	0	-	-	-	
Hydrothorax	2	33%	4	67%	1.60 (0.28–9.17)	0.595	[d]	0	-	0	-	-	-	
Convulsions	0	0%	3	100%	-	0.322	[d]	0	-	0	-	-	-	
Encephalopathy	3	75%	1	25%	10.24 (1.03–102.2)	0.016	[d]	1	100%	0	0%	-	0.172	[d]
Hypotonia/feeding difficulties	3	75%	1	25%	10.24 (1.03–102.2)	0.016	[d]	1	100%	0	0%	-	0.172	[d]
Limb anomaly	1	25%	3	75%	6.60 (0.58–75.34)	0.083	[d]	0	-	0	-	-	-	
Dysmaturity	0	0%	3	100%	-	0.322	[d]	0	-	0	-	-	-	
Clinical presentation														
Cardiac anomaly	13	21%	50	79%	0.68 (0.31–1.53)	0.355	[c]	2	11%	16	89%	0.33 (0.05–2.42)	0.264	[d]
Genitourinary anomaly	1	9%	10	91%	0.29 (0.04–2.36)	0.294	[d]	0	0%	3	100%	-	1.000	[d]
Brain anomaly	11	28%	28	72%	1.18 (0.47–2.99)	0.724	[c]	3	33%	6	67%	4.50 (0.60–33.71)	0.287	[d]
Seizures	1	13%	7	88%	0.43 (0.05–3.62)	0.679	[d]	0	0%	1	100%	-	1.000	[d]
Eye anomaly	1	14%	6	86%	0.51 (0.06–4.36)	1.000	[d]	0	-	0	-	-	-	
Dysmorphic features	23	30%	54	70%	2.18 (0.92–5.17)	0.074	[c]	3	18%	14	82%	1.07 (0.15–7.64)	1.000	[d]
Other anomalies	24	27%	64	73%	1.68 (0.69–4.14)	0.251	[c]	15	79%	4	21%	2.40 (0.23–24.96)	0.633	[d]
Number anomalies (all)														
≥2	24	26%	70	74%	1.29 (0.52–3.19)	0.587	[c]	4	17%	19	83%	1.05 (0.10–11.63)	1.000	[d]
≥3	11	25%	33	75%	1.06 (0.46–2.46)	0.886	[c]	2	18%	9	82%	1.11 (0.16–7.97)	1.000	[d]

MCA multiple congenital anomalies.
[a]Exome sequencing diagnoses made after a single gene was analyzed were excluded.
[b]Only categories reported >1 time were included.
[c]Chi-square.
[d]Fisher's exact test.

Table 4 Overview of the calculation of the yield of unsuspected diagnoses in sequencing studies performed in NICU infants

Study	Total patients	Diagnosed	Unsuspected diagnoses	Undiagnosed	Total yield	Yield unsuspected diagnoses
Willig et al.[2]	35	20	9	15	57.1%	37.5%
Daoud et al.[7]	20	8	6	12	40.0%	33.3%
Van Diemen et al.[4]	23	7	6	16	30.4%	27.3%
Meng et al.[3a]	278	102	39	170	36.7%	18.7%
This study[b]	31	7	5	24	22.6%	17.2%

Willig et al.[2] (2015) and Van Diemen et al.[4] (2017) performed rapid genome sequencing; Daoud et al.[7] (2016) used a gene panel; Meng et al.[3] (2017) performed regular and rapid exome sequencing; this study performed only regular exome sequencing.
NICU neonatal intensive care unit.
[a]An additional six patients were diagnosed retrospectively with myotonic dystrophy, infantile botulism, and four partial diagnoses.
[b]Exome sequencing patients only.

patients and the fact that our cohort is limited to NICU or PICU infants. It is possible that the admission to NICU/PICU in itself significantly raises the possibility of a genetic diagnosis and that therefore the effect of the number of reported features cannot be ascertained with our sample size.

Consequences of genetic diagnosis

In almost all patients, the genetic diagnosis had consequences (90.6%, 29/32), and this remains high when excluding patients only receiving parental reproductive counseling, prognostic information, or future screenings (68.8%, 22/32). The clinical effect of all genetic diagnoses (68.8%) is comparable with other NICU studies (Willig et al.[2] 13/20, 65.0%; Daoud et al.[7] 2/8, 25.0%; Meng et al.[3] 53/102, 52.0%; Van Diemen et al.[4] 5/7, 71.4%).

One aspect that is difficult to quantify is the reduction in burden and costs of additional investigations because of a genetic diagnosis. For example, in patient 22, who presented with severe feeding difficulties, regular care would have included extensive swallowing tests. In other patients, metabolic testing and biopsies might have been performed in absence of a genetic diagnosis. Unfortunately, the retrospective study design does not lend itself to accurately quantify these aspects.

Exome sequencing or genome sequencing

Most other NICU studies have used rGS rather than rES. Although ES capture protocols are being refined and optimized, the capture step still takes additional time compared with GS, as evidenced from the faster turnaround times (2–7 days versus 7–14 days) in GS studies. There are other advantages of GS, of which the ability to detect small copy-number changes is clinically the most relevant. On the other hand, ES has a higher coverage and is therefore more suitable to detect postzygotic mosaicism.[9] Intronic and intergenic coverage do not seem to raise diagnostic yield currently, because previous studies have shown that GS application almost uniquely leads to exonic diagnoses.[10–13] Despite recent reductions in sequencing costs, ES is still more cost-effective and more widely available than GS,[14] and considering our limited financial resources we have chosen to implement a rES protocol in September 2016. When speed is truly of the essence, rGS is preferable.

Conclusion: what is the place of (r)ES/GS in the NICU?

The data reported in this paper provide a first estimate of the (additional) yield of ES in the NICU. The total yield of ES in our study population is 17%, and although this seems much lower than the previously reported NICU yields, it is actually comparable with the "unsuspected yields" of previous papers (Table 4). Our study shows that the yield of the classical genetic approach is high, and ES should not be performed without prior genetic consultation. This will not only reduce costs, but also increase ES yield as evidenced from several patients in our study where ES was initially negative. Additionally, some diagnoses could not have been obtained from ES in blood, such as the low mosaic variant in *PIK3CA* in patient 23, which was only detected in fibroblasts.

It could be argued that a fast diagnosis may be helpful to parents and prevent an extensive diagnostic odyssey. Although this is certainly a valid reason, in a budget-restrictive health-care system spending money on rapid sequencing protocols (which can be up to twice as expensive as regular sequencing due to lower volumes) means that other procedures cannot be performed. We expect that regular turnaround times of ES will decrease significantly in the coming years, reducing the need for bespoke rapid protocols, while also providing parents with a result in a timely manner.

In conclusion, our retrospective study shows that although ES has a place in NICU diagnostics, its yield may not be as spectacular as indicated by early studies, mostly because many diagnoses may also be obtained by alternative, and much cheaper, strategies in a similar timeframe.

ELECTRONIC SUPPLEMENTARY MATERIAL

The online version of this article (https://doi.org/10.1038/s41436-018-0293-0) contains supplementary material, which is available to authorized users.

DISCLOSURE

The authors declare no conflicts of interest.

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