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Economische evaluaties van exoomsequencing bij zeldzame aandoeningen binnen de kindergeneeskunde

(met een samenvatting in het Nederlands)

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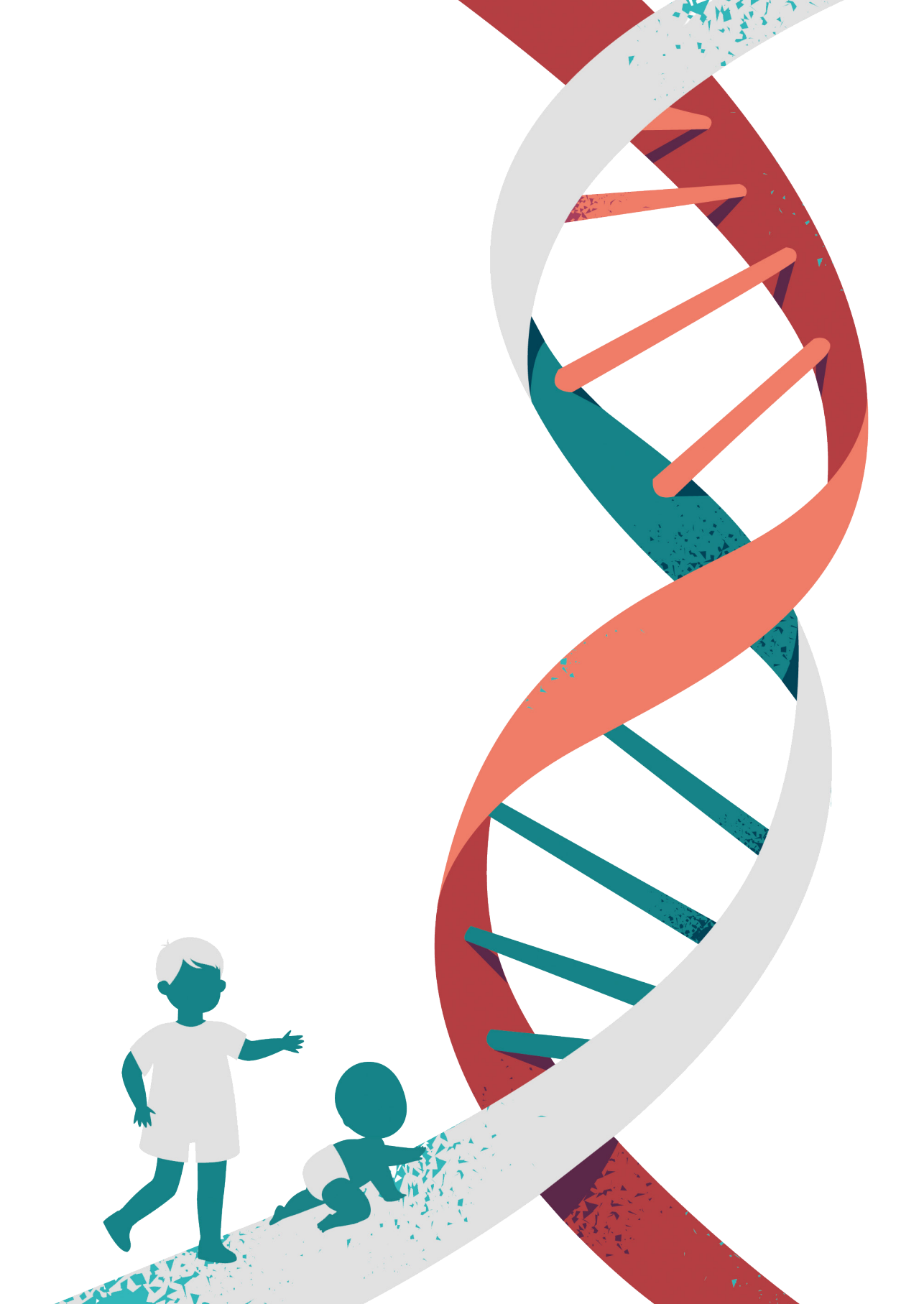


CHAPTER 1

General introduction

1.1 GENETIC DIAGNOSIS OF RARE DISEASES

World-wide, one in 17 individuals is affected by a rare disease; generally meaning a disease that affects fewer than 1 in 2,000 people [1]. Currently, there are 7,000 rare diseases known. For approximately 75% of these diseases, the first clinical symptoms manifest during (early) childhood, and 30% of children with a rare disease die before the age of 5 [2-8]. The vast majority of rare diseases (~80%) has an underlying genetic etiology [9, 10]. Obtaining this genetic diagnosis for rare diseases is, however, complex due to extensive genetic and clinical heterogeneity of these disorders. This results in individuals with a rare disease being referred to different medical specialists, laboratories and subsequently multiple genetic diagnostic tests are required before the molecular diagnosis is identified [11]. A trajectory that is thus unpredictable, uncertain, often long and costly, and perceived as 'a diagnostic odyssey'. Moreover, a conclusive molecular diagnosis is only obtained in a fraction of the individuals [11]. This creates an impasse, as the field of medical genetics considers a fast and accurate genetic diagnosis to be the starting point for personalized medicine, i.e. to guide decisions on prevention, treatment and care.

Improving the diagnostic trajectory to rapidly identify a genetic diagnosis may thus impact clinical decisions of patients with a rare disorder. With many of the rare diseases presenting in the first 28 days of life, it may be well hypothesized that newborns admitted to a neonatal intensive care unit (NICU) make an excellent use case to show the importance and impact of a fast and reliable genetic diagnosis. That is, a large part of newborns admitted to a NICU may have a genetic disorder, and their diagnostic trajectories is extensive with high healthcare utilization [12]. This has a negative impact on clinical, economic and personal aspects. For patients admitted to the NICU, the time-consuming diagnostic trajectory itself can be long and burdensome for the (parents of the) patient, and not having a conclusive diagnosis can lead to a lot of uncertainties. In addition, in absence of a diagnosis, it is difficult to give an accurate prognosis of survival and outcomes, which can be burdensome for patients and their parents. Furthermore, a diagnosis might impact clinical decision-making, for example by starting treatment, or by preventive measures to reduce health risk and symptoms of the disease. In addition, the psychosocial impact a diagnosis has on (parents of) patients should not be underestimated, as they can search for social support networks [13, 14].

1.2 GENETIC ASSAYS TO DIAGNOSE RARE DISEASES

To diagnose rare diseases, there are several complementary cytogenetic and molecular diagnostic technologies to provide genetic diagnoses (Table 1), with two aspects of genetic testing mostly discriminating them. The first is the scope of testing, being either 'genome-wide', looking at all of the genome, such as for karyotyping or genome-wide microarrays, or alternatively, 'targeted', assessing only a small portion of it, such as the case for fluorescence in situ hybridization (FISH) or polymerase chain reaction (PCR)-based methods. The second is the resolution of the technology, being either 'low', such as chromosomes being visible under a light microscope, or 'high' Sanger sequencing, referring to the DNA sequence being read at single nucleotide resolution. Ideally, technologies combine a high resolution, with a genome-wide view.

Over the last decade, technological innovations, collectively termed next-generation sequencing (NGS) technologies, have made it possible to combine such genome-wide view with high resolution, allowing to map out an individual's entire DNA sequence at base pair level [15]. This has had major impact within the field of medical genetics and pediatrics including the NICU. With the introduction of NGS, it was no longer needed to, based on a patient's clinical presentation, a priori select a region of the genome for DNA analysis (e.g. one gene) to find an underlying genetic defect explaining disease. Instead, NGS allows an unbiased DNA analysis (e.g. all 21,000 human genes at once) [15, 16]. The latter is of particular importance for genetically and clinically heterogeneous disorders, as the disease could be caused by a single DNA error in one of as much as 1,500 different genes without the phenotype of the patient being specific enough to pinpoint which of these 1,500 genes to test for (e.g. for neurodevelopmental disorders).

In 2012, exome sequencing (ES), a form of NGS, was introduced in the clinic, enabling sequencing all protein-coding genes at once [17]. Another development within the field of genetics was the introduction of genome sequencing (GS). In contrast to ES, GS also analyses the non-coding DNA-sequences, i.e. the entire human genome [18]. The resolution of both ES and GS is much larger compared to other technologies (Table 1). Another major advantage of both ES and GS is that no a-priori knowledge about the underlying disease is necessary [18]. The introduction of ES and GS creates a shift from a phenotype-first approach to a genotype-first approach.

Table 1. Overview of (previously) most commonly used genetic assays to diagnose rare genetic disorders

Technology	Genome wide / Targeted	Type of variant detectable	Resolution (size detection limit)	Example(s)
Karyotyping	Genome wide	(Un)balanced translocations; aneuploidies; microscopically visible gains and losses	>5 Mb	Robertsonian translocation; t(2;X)(p22.3;q28); 4p-
Genomic microarrays	Genome wide	Aneuploidies, submicroscopic gains and losses (CNVs)	>10 kb*	Koolen-de Vries syndrome (17q21.31)
QF-PCR	Targeted- multi locus	Trisomy/monosomy 13, 18, 21, and sex chromosomes	Numerical whole chromosome abnormalities**	Down syndrome (trisomy 21)
FISH	Targeted- single/ multi locus	Structural abnormalities, confirmation of CNVs, translocations, numerical abnormalities	200-300 kb**	Subtelomere FISH for neurodevelopmental syndrome
Repeat primed PCR	Targeted- single locus	STR length determination	Number of repeats present in sample	Fragile X syndrome (*FMR1, (CGG)*n repeat)
Methylation assay	Targeted- single locus	Methylation defects	Locus specific	Prader Willi/ Angelman syndrome
Sanger sequencing	Targeted- single locus	SNVs, insertion-deletion events	Base pair level	CHARGE syndrome (*CHD7)*
MLPA	Targeted- single locus	CNVs	Single exon level***	CHARGE syndrome (*CHD7)*
ES	Targeted- all protein coding	CNVs, numerical variants, SNVs, insertion-deletion events, STRs in coding sequence	Base pair level for SNV/indel, CNVs at (single) exon level	All clinically and genetically heterogeneous rare genetic disease
GS	Genome wide	CNVs, numerical variants, SNVs, insertion-deletion events, STRs, and all other structural variations such as inversions and translocations.	Base pair level	All clinically and genetically heterogeneous rare genetic disease

* Determined by the type of microarray used and number of probes per distance. Here: CytoScan high-density; ** Determined by the type probe, and probe spacing used; *** Determined by the number of probes per distance. Often used for single exon deletion/duplication testing in combination with Sanger sequencing for targeted disease genes. QF-PCR = quantitative fluorescent polymerase chain reaction; FISH = fluorescence in situ hybridization; PCR = polymerase chain reaction; MLPA = multiplex ligation-dependent probe amplification; CNV = copy number variation; SNV = single nucleotide variant; STR = short tandem repeat.

1.3 EXPLOITING THE ADVANTAGES OF ES AND GS

Exome sequencing and genome sequencing are -per test- perceived to be more expensive than the techniques previously used, but they allow to combine the advantage of genome wide analysis with the ultimate resolution at base pair level. Besides these technical advantages of ES or GS, there are also other possible advantages of ES or GS used to confirm a genetic diagnosis in children with suspected genetic condition:

Reducing complexity and length of the diagnostic trajectory
One example of clinical benefits of implementing ES or GS is the impact on complexity of the genetic diagnostic trajectory. Instead of performing multiple (consecutive or parallel) genetic tests, just one single test, i.e. ES or GS, might be sufficient in order to provide the molecular diagnosis, which can reduce the complexity of the genetic diagnostic trajectory. As a consequence, the time-to-diagnosis is also expected to be shortened [19-21]. The latter is even more evident if ES or GS is performed as first-tier, and rapid test (rapid ES or rapid GS), providing the diagnostic outcome of genetic testing in 5-14 days.

Increasing the diagnostic yield
The use of multiple complementary genetic assays is chosen to account for the genetic and clinical heterogeneity. Yet, it is practically beyond reach to test for all genes, and all variant types. Consequently, the overall diagnostic yield obtained in a cohort of patients with rare disease, is still low (5-20%). Based on the size detection limit and detectable type of variants of ES and GS (Table 1), implementing these technologies instead of routine genetic testing might impact the efficiency of the diagnostic trajectory, and results in an increased diagnostic yield [19-21].

Reducing burden of the diagnostic trajectory
As mentioned, the diagnostic trajectory can be burdensome for both patients and their parents. By ending the diagnostic odyssey, i.e. by aiming at shortening the length of the diagnostic trajectory and increasing diagnostic yield by implementing ES or GS, experienced burden might be reduced. Moreover, research has shown that implementation of ES in rapid mode, i.e. rapid ES, is clinically effective for patients admitted to the NICU or pediatric intensive care unit (PICU), but also relieves the burden of stress and uncertainty in families [2, 22].

Supporting clinical decision-making
Based on early diagnosis, treatment can be started (earlier) or ineffective or harmful therapies can be stopped. Furthermore, complications of the disease can be prevented or specialists can anticipate on expected complications by long-term strategies, e.g.

by surgery or rehabilitation. A genetic diagnosis can also guide genetic counseling for parents of the patient or it can assist in the discussion regarding end-of-life decisions.

1.4 COST-EFFECTIVENESS OF GENETIC DIAGNOSTICS

The implementation of a new technology, such as ES or GS, does not only involve clinical or ethical considerations. The economic impact should also be taken into account. As mentioned, in order to diagnose a patient with a rare disease of presumed genetic origin, so far, often multiple tests are used, questioning whether the overall costs to diagnose a patient by the new techniques, i.e. ES or GS, are also more expensive. Cost-effectiveness plays a crucial role regarding the implementation of a new technology and depends on both the costs and clinical benefit [23]. In the ideal scenario, a new technology should cost less (or be cost neutral) compared to the alternative, or currently used technology, and/or should positively affect clinical outcomes in order to be cost-effective. In practice, a cost-effectiveness analysis (CEA) is performed where the relationship between at least two technologies or interventions and their effect on health outcomes is investigated and can be used to inform policymakers about making decisions regarding reimbursement and implementation of the new technology [1, 24, 25].

General guidelines for performing a CEA

Before performing a CEA, it is important to know which outcome measurements should be used. According to the National Institute for Health and Clinical Excellence (NICE) and Zorginstituut Nederland (ZIN), cost per quality-adjusted life year (QALY) is the preferred outcome measure [24, 26, 27]. A QALY represents one extra year of life in perfect health and takes into account both the quantity and quality of life in a certain health state [25, 28]. QALYs are assessed by using questionnaires about different dimensions of daily life, for example the EuroQol-5D (EQ-5D-5L) [23].

Based on the incremental cost-effectiveness ratio (ICER), a policymaker can decide whether a new intervention or technology should be implemented in local or national policies [23, 24]. An ICER compares two different interventions or technologies and is based on the incremental health outcomes and incremental costs. For example when comparing two interventions (Table 2), i.e. the currently used treatment (O) compared to a new treatment (A). Both interventions are related to a certain health outcome, in this case the QALY. The incremental health outcome is defined as the difference in health outcome for the two interventions and can be calculated by subtracting the health benefit of O (i.e. 0.7) by the health benefit of A (i.e. 0.9). Incremental costs are defined by the difference in costs for the currently used technology (O) and the new technology (A).

Table 2. Example of comparing two different treatment strategies

	Costs	Health benefit (QALY)
Currently used treatment (O)	€700	0.7
Alternative treatment (A)	€500	0.9

QALY = quality-adjusted life years gained

The ICER can be calculated by dividing the incremental costs by the incremental health outcomes ($\frac{(Costs\ A - Cost\ O)}{(QALY\ A - QALY\ O)} = ICER$). In the example provided in Table 2, this would result in (€500-€700)/(0.9-0.7) = -€1,000. This means that implementing the new intervention will save €1,000 per QALY gain. A negative ICER indicates that the new technology results in a decrease in costs, improvement of health outcomes or both compared to the current technology (Figure 1), suggesting that it is recommended to implement the new technology [1].

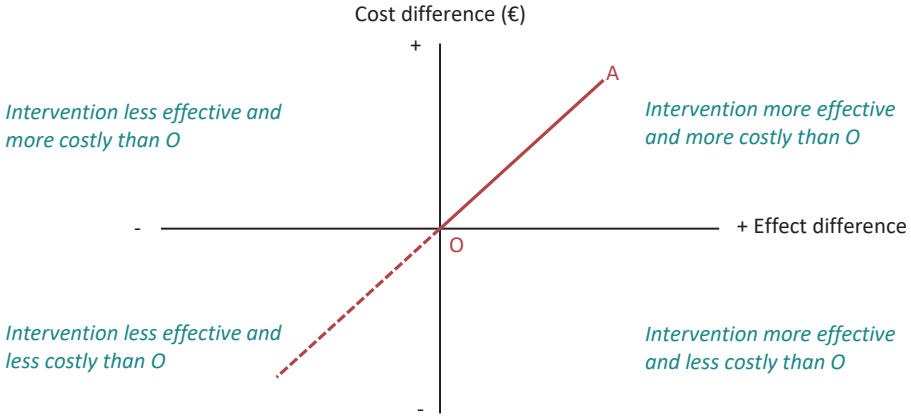


Figure 1. Cost-effectiveness plane, based on Bick and Dimmock [29].
The red line represents the change in costs and effects for the currently used technology, intervention or treatment (O) and the alternative technology, intervention or treatment (A).

Guidelines for performing a CEA within genetics

Within the field of genetics, it might be difficult to use cost per QALY as outcome measure, since QALYs are mainly based on patients' quantified perception of wellbeing. Especially with children or infants as target population, it can be quite challenging to get a realistic representation of QALYs. Furthermore, QALYs are also determined only for directly affected patients instead of taking relatives into account, although earlier research has shown that genetic information is also very important for relatives due to

potential harm or benefit of a genetic disease for family members [23, 30, 31].

Within pediatric genetics, there are guidelines for performing economic evaluations. However, according to earlier research there is a lack of adherence to these existing guidelines and there is need for additional guidance to assist clinical decision-making [32]. At the moment, it is unclear which outcome measurements should be included in a CEA focused on NGS innovations for rare disease diagnostics, and what the time horizon, i.e. time period over which costs are collected, should look like.

Current cost-effectiveness evidence

Regarding the cost-effectiveness of implementing ES or GS, scientific evidence is relatively scarce [33]. By ending the diagnostic odyssey, future healthcare resource use and costs can be prevented. A previous study showed that an earlier diagnosis, especially with improved genetic testing, will positively affect the diagnostic odyssey and significantly reduce costs associated with this diagnostic trajectory [34]. However, an earlier diagnosis can also lead to an earlier start of treatment, also preventing future healthcare costs, but may in fact also lead to increase in costs because of the use genetic therapies, which without knowledge of the diagnosis, may not have spent [17, 35]. The latter will, however, only become visible if CEAs include a longer time window than up until the ES diagnostic outcome. Although implementation of ES or GS might be cost-effective in the future for the diagnostic trajectory, more research is needed to support the implementation of ES or GS [2, 36-38]. It is unclear what differences in costs related to the genetic diagnostic trajectory can be expected.

Within the Netherlands, several studies have been performed on the clinical utility and economic impact of ES compared to current genetic testing [36, 39]. These studies suggest that implementation of ES might be clinically beneficial and cost-effective in patient care. However, it remains challenging to estimate the impact in case ES is implemented as first-tier test.

1.5 RESEARCH QUESTIONS

In the last decade, the landscape of genetic testing has changed enormously due to the development and introduction of NGS technologies. ES has found a stable position in identifying the genetic etiology of rare disease, and increased diagnostic yield. There is however still an unmet need to better understand the economic impact of ES implementation in routine care, also including the other possible benefits of ES, such as improvements in clinical decision-making, and reduced burden to families. A cohort of patients in which these economic outcomes might be best captured are neonates

admitted to Dutch NICUs. For these neonates, it is expected that genetic tests results may impact clinical decision-making, yet, genetic testing using ES has not reached widespread implementation. The latter is mainly due to the need of NICUs to have a fast ES result, requiring infrastructure on Dutch genetic laboratories. Since there are also still uncertainties regarding the values to be taking into account for economic evaluation when implementing novel genetic tests. Hence, the overall aim of this thesis is to assess the economic value of implementing ES compared to routine genetic diagnostic testing within pediatric care. Hereto, the following research questions will be addressed:

1. What are the best approaches for economic evaluations of next generation sequencing in pediatric care, what are the included costs, effects and time horizons?
2. What are the average healthcare costs for patients admitted to the NICU, and what is the role of genetic testing in this trajectory?
3. What is the impact of implementing (rapid) ES on these total healthcare costs?

In order to assess the added value of implementing ES, it is important to know what to include in an economic evaluation. Therefore, **chapter 2** reviews the included costs, effects and time horizons of studies on implementation of ES and/or GS in pediatric genetics to deduce a framework for future economic evaluations in a changing genetic diagnostic landscape.

In **chapter 3**, a single-center retrospective observational cohort study is described of neonates admitted to the NICU, including a clinical characterization of the cohort, as well as the uptake of genetic testing, and diagnostic yield obtained. Based on these results, strategic options were suggested to maximize diagnostic yield by implementing rapid ES. The healthcare costs and genetic testing of this cohort are explored in **chapter 4**. Furthermore, this chapter includes four scenarios in which the economic consequences of implementing ES was modelled.

Chapter 5 details the clinical and economic consequences of implementing ES at the NICU as first-tier test in neonates, by a national prospective multicenter clinical utility study. All neonates received both the traditional genetic diagnostic trajectory and the genetic trajectory in which rapid ES was performed, allowing for a direct comparison of outcome measures including diagnostic yield, and economic consequences.

Chapter 6 summarizes the studies of this thesis and provides an overall reflection on results obtained, and places these in a context for future economic evaluations of novel technologies in the field of medical genetics.

REFERENCES

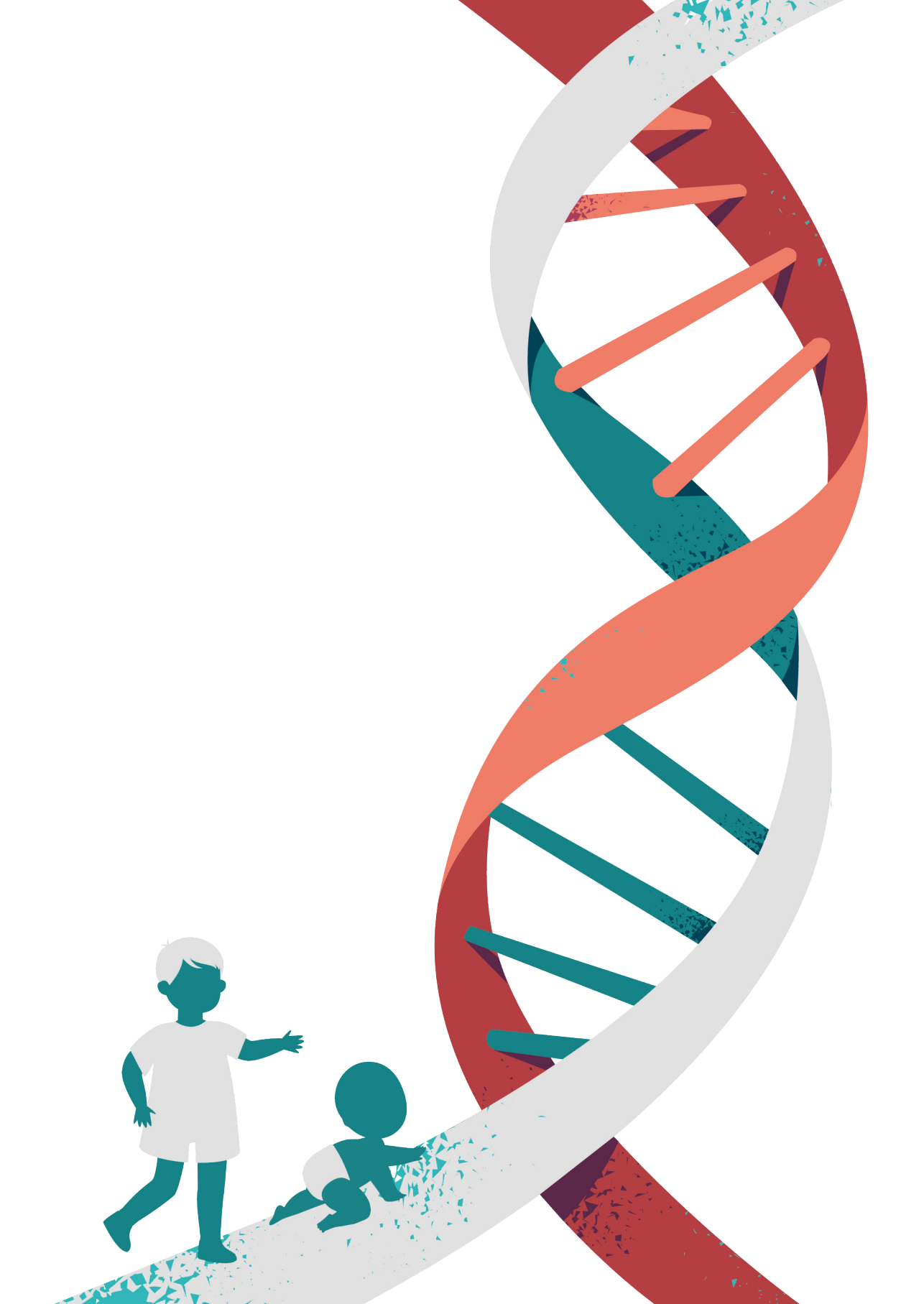
1. Drummond, M.F., et al., Methods for economic evaluation of health care programmes. 4 ed. 2015, Oxford: Oxford University Press.
2. Borghesi, A., Mencarelli, M. A., Memo, L., Ferrero, G. B., Bartuli, A., Genuardi, M., Stronati, M., Villani, A., Renieri, A., & Corsello, G. (2017). Intersociety policy statement on the use of whole-exome sequencing in the critically ill newborn infant. Italian Journal of Pediatrics, 43(1). https://doi.org/10.1186/s13052-017-0418-0.
3. Costa, S., Rodrigues, M., Centeno, M. J., Martins, A., Vilan, A., Brandão, O., & Guimarães, H. (2010). Diagnosis and cause of death in a neonatal intensive care unit - How important is autopsy? Journal of Maternal-fetal & Neonatal Medicine, 24(5), 760-763. https://doi.org/10.3109/14767058.2010.520047.
4. Simpson, C., Ye, X. Y., Hellmann, J., & Tomlinson, C. (2010). Trends in Cause-Specific mortality at a Canadian outborn NICU. Pediatrics, 126(6), e1538–e1544. https://doi.org/10.1542/peds.2010-1167.
5. Tyson, J. E., & Kennedy, K. A. (2002). Variations in mortality rates among Canadian neonatal intensive care units: interpretation and implications. PubMed, 166(2), 191–192. https://pubmed.ncbi.nlm.nih.gov/11826942.
6. Weiner, J., Sharma, J., Lantos, J. D., & Kilbride, H. W. (2011). How infants die in the Neonatal Intensive Care Unit. Archives of Pediatrics & Adolescent Medicine, 165(7), 630. https://doi.org/10.1001/archpediatrics.2011.102.
7. Wen, S. W., Liu, S., Joseph, K. S., Rouleau, J., & Allen, A. C. (2000). Patterns of infant mortality caused by major congenital anomalies. Birth Defects Research, 61(5), 342–346. https://doi.org/10.1002/(sici)1096-9926(200005)61:5.
8. Wojcik, M. H., Schwartz, T. S., Yamin, I., Edward, H. L., Genetti, C. A., Towne, M. C., & Agrawal, P. B. (2018). Genetic disorders and mortality in infancy and early childhood: delayed diagnoses and missed opportunities. Genetics in Medicine, 20(11), 1396–1404. https://doi.org/10.1038/gim.2018.17.
9. European Commission. Rare diseases. Available from: https://ec.europa.eu/info/research-and-innovation/research-area/health-research-and-innovation/rare-diseases_en.
10. Boycott, K. M., Vanstone, M. R., Bulman, D. E., & MacKenzie, A. (2013). Rare-disease genetics in the era of next-generation sequencing: discovery to translation. Nature Reviews Genetics, 14(10), 681–691. https://doi.org/10.1038/nrg3555.
11. Shashi, V., McConkie-Rosell, A., Rosell, B., Schoch, K., Vellore, K., McDonald, M., Jiang, Y., Xie, P., Need, A. C., & Goldstein, D. B. (2014). The utility of the traditional medical genetics diagnostic evaluation in the context of next-generation sequencing for undiagnosed genetic disorders. Genetics in Medicine, 16(2), 176–182. https://doi.org/10.1038/gim.2013.99.
12. Wu, A. C., McMahon, P. M., & Lu, C. Y. (2020). Ending the diagnostic Odyssey—Is Whole-Genome sequencing the answer? JAMA Pediatrics, 174(9), 821. https://doi.org/10.1001/jamapediatrics.2020.1522.
13. Horton, R., & Lucassen, A. (2019). Recent developments in genetic/genomic medicine. Clinical Science, 133(5), 697–708. https://doi.org/10.1042/cs20180436.
14. Rosenthal, E. T., & Biesecker, L. G. (2001). Parental attitudes toward a diagnosis in children with unidentified multiple congenital anomaly syndromes. American Journal of Medical Genetics, 103(2), 106–114. https://doi.org/10.1002/ajmg.1527.
15. Majewski, J., Schwartzentruber, J., Lalonde, E., Montpetit, A., & Jabado, N. (2011). What can exome sequencing do for you? Journal of Medical Genetics, 48(9), 580–589. https://doi.org/10.1136/jmedgenet-2011-100223.
16. Gilissen, C., Hoischen, A., Brunner, H. G., & Veltman, J. A. (2012). Disease gene identification strategies for exome sequencing. European Journal of Human Genetics, 20(5), 490–497. https://doi.org/10.1038/ejhg.2011.258.
17. Choi, M., Scholl, U. I., Ji, W., Liu, T., Tikhonova, I., Zumbo, P., Nayır, A., Bakkaloğlu, A., Özen, S., Sanjad, S. A., Nelson-Williams, C., Farhi, A., Mane, S., & Lifton, R. P. (2009). Genetic diagnosis by whole exome capture and massively parallel DNA sequencing. Proceedings of the National Academy of Sciences of the United States of America, 106(45), 19096–19101. https://doi.org/10.1073/pnas.0910672106.
18. Carroll, J., Wigby, K., & Murray, S. (2020). Genetic testing strategies in the newborn. Journal of Perinatology, 40(7), 1007–1016. https://doi.org/10.1038/s41372-020-0697-y.
19. Vrijenhoek, T., Kraaijeveld, K., Elferink, M., De Ligt, J., Kranendonk, E., Santen, G. W., Nijman, I. J., Butler,

D., Claes, G. R. F., Costessi, A., Dorlijn, W., Van Eyndhoven, W., Halley, D., Van Den Hout, M. C. G. N., Van Hove, S., Johansson, L., Jongbloed, J. D. H., Kamps, R., Kockx, C., . . . Cuppen, E. (2015). Next-generation sequencing-based genome diagnostics across clinical genetics centers: implementation choices and their effects. European Journal of Human Genetics, 23(9), 1142–1150. https://doi.org/10.1038/ejhg.2014.279.

20. Koboldt, D. C., Steinberg, K. M., Larson, D. E., Wilson, R. K., & Mardis, E. R. (2013). The Next-Generation Sequencing Revolution and its impact on genomics. Cell, 155(1), 27–38. https://doi.org/10.1016/j.cell.2013.09.006.
21. Klee, E. W., Cousin, M. A., Vairo, F. P. E., Morales-Rosado, J. A., Macke, E. L., Jenkinson, W. G., Ferrer, A., Schultz-Rogers, L., Olson, R. J., Oliver, G. R., Sigafoos, A. N., Schwab, T. L., Zimmermann, M. T., Urrutia, R., Kaiwar, C., Gupta, A., Blackburn, P. R., Boczek, N. J., Prochnow, C. A., . . . Lazaridis, K. N. (2021). Impact of integrated translational research on clinical exome sequencing. Genetics in Medicine, 23(3), 498–507. https://doi.org/10.1038/s41436-020-01005-9.
22. Śmigiel, R., Biela, M., Szmyd, K., Błoch, M., Szmida, E., Skiba, P., Walczak, A., Gasperowicz, P., Kosińska, J., Rydzanicz, M., Stawiński, P., Biernacka, A., Zielińska, M., Gołebiowski, W., Jalowska, A., Ohia, G., Głowska, B., Walas, W., Królak-Olejnik, B., . . . Płoski, R. (2020). Rapid Whole-Exome sequencing as a diagnostic tool in a Neonatal/Pediatric Intensive Care unit. Journal of Clinical Medicine, 9(7), 2220. https://doi.org/10.3390/jcm9072220.
23. Grosse, S. D., Wordsworth, S., & Payne, K. (2008). Economic methods for valuing the outcomes of genetic testing: beyond cost-effectiveness analysis. Genetics in Medicine, 10(9), 648–654. https://doi.org/10.1097/gim.0b013e3181837217.
24. McCabe, C., Claxton, K., & Culyer, A. J. (2008). The NICE Cost-Effectiveness Threshold. PharmacoEconomics, 26(9), 733–744. https://doi.org/10.2165/00019053-200826090-00004.
25. Brooke, B. S., Kaji, A. H., & Itani, K. M. (2020). Practical Guide to Cost-effectiveness Analysis. JAMA Surgery, 155(3), 250. https://doi.org/10.1001/jamasurg.2019.4392.
26. National Institute for Health and Care Excellence (NICE, 2013). Guide to the methods of technology appraisal. Available from: https://www.nice.org.uk/process/pmg9/resources/guide-to-the-methods-of-technology-appraisal-2013-pdf-2007975843781.
27. Zorginstituut Nederland (ZIN, 2019). Kostenhandleiding: Methodologie van kostenonderzoek en referentieprijzen voor economische evaluaties in de gezondheidszorg. Available from: https://www.zorginstituutnederland.nl/binaries/zinl/documenten/publicatie/2016/02/29/richtlijn-voor-het-uitvoeren-van-economische-evaluaties-in-de-gezondheidszorg/Richtlijn+voor+het+uitvoeren+van+economische+evaluaties+in+de+gezondheidszorg+%28verdiepingsmodules%29.pdf15-12.
28. Weinstein, M. C., Torrance, G. W., & McGuire, A. (2009). QALYs: The Basics. Value in Health, 12, S5–S9. https://doi.org/10.1111/j.1524-4733.2009.00515.x.
29. Bick, D., & Dimmock, D. (2011). Whole exome and whole genome sequencing. Current Opinion in Pediatrics, 23(6), 594–600. https://doi.org/10.1097/mop.0b013e32834b20ec.
30. Neumann, P. J., Goldie, S. J., & Weinstein, M. C. (2000). Preference-Based measures in economic evaluation in health care. Annual Review of Public Health, 21(1), 587–611. https://doi.org/10.1146/annurev.publhealth.21.1.587.
31. McAllister, M., Payne, K., Nicholls, S. G., MacLeod, R., Donnai, D., & Davies, L. (2007). Improving service evaluation in Clinical genetics: Identifying effects of genetic diseases on individuals and families. Journal of Genetic Counseling, 16(1), 71–83. https://doi.org/10.1007/s10897-006-9046-3.
32. Alam, K., & Schofield, D. (2018). Economic evaluation of genomic sequencing in the paediatric population: a critical review. European Journal of Human Genetics, 26(9), 1241–1247. https://doi.org/10.1038/s41431-018-0175-6.
33. Schwarze, K., Buchanan, J., Taylor, J. C., & Wordsworth, S. (2018). Are whole-exome and whole-genome sequencing approaches cost-effective? A systematic review of the literature. Genetics in Medicine, 20(10), 1122–1130. https://doi.org/10.1038/gim.2017.247.
34. Gonzaludo, N., Belmont, J. W., Gainullin, V. G., & Taft, R. J. (2019). Estimating the burden and economic impact of pediatric genetic disease. Genetics in Medicine, 21(8), 1781–1789. https://doi.org/10.1038/s41436-018-0398-5.
35. Kim, J., Hu, C., Achkar, C. M. E., Black, L. E., Douville, J., Larson, A., Pendergast, M. K., Goldkind, S. F., Lee, E. A., Kuniholm, A., Soucy, A., Vaze, J., Belur, N. R., Fredriksen, K., Stojkovska, I., Tsytsykova, A. V., Armant, M., DiDonato, R. L., Choi, J. Y., . . . Yu, T. W. (2019). Patient-Customized oligonucleotide therapy for a rare

genetic disease. The New England Journal of Medicine, 381(17), 1644–1652. https://doi.org/10.1056/nejmoa1813279.

36. Vissers, L. E., Van Nimwegen, K., Schieving, J., Kamsteeg, E. J., Kleefstra, T., Yntema, H. G., Pfundt, R., Van Der Wilt, G. J., Brunner, H. G., Van Der Burg, S., Grutters, J. P., Veltman, J. A., & Willemsen, M. (2017). A clinical utility study of exome sequencing versus conventional genetic testing in pediatric neurology. Genetics in Medicine, 19(9), 1055–1063. https://doi.org/10.1038/gim.2017.1.
37. Smith, H. S., Swint, J. M., Lalani, S. R., Yamal, J., De Oliveira Otto, M. C., Castellanos, S., Taylor, A., Lee, B., & Russell, H. V. (2019). Clinical Application of Genome and Exome Sequencing as a Diagnostic Tool for Pediatric Patients: a Scoping Review of the Literature. Genetics in Medicine, 21(1), 3–16. https://doi.org/10.1038/s41436-018-0024-6.
38. Howell, K., Eggers, S., Dalziel, K., Riseley, J. R., Mandelstam, S., Myers, C. T., McMahon, J. M., Schneider, A., Carvill, G. L., Mefford, H. C., Scheffer, I. E., & Harvey, A. S. (2018). A population-based cost-effectiveness study of early genetic testing in severe epilepsies of infancy. Epilepsia, 59(6), 1177–1187. https://doi.org/10.1111/epi.14087.
39. Vrijenhoek, T., Middelburg, E. M., Monroe, G. R., Van Gassen, K., Geenen, J. W., Hövels, A. M., Knoers, N. V. a. M., Van Amstel, H. K. P., & Frederix, G. W. (2018). Whole-exome sequencing in intellectual disability; cost before and after a diagnosis. European Journal of Human Genetics, 26(11), 1566–1571. https://doi.org/10.1038/s41431-018-0203-6.



CHAPTER 2

Economic evaluations of exome and genome sequencing in pediatric genetics: considerations towards a consensus strategy

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ABSTRACT

Next Generation Sequencing (NGS) is increasingly used for the diagnosis of rare genetic disorders. The aim of this study is to review the different approaches for economic evaluations of NGS in pediatric care used to date, to identify all costs, effects and time horizons taken into account. A systematic literature review was conducted to identify published economic evaluations of NGS applications in pediatric diagnostics, i.e. exome sequencing (ES) and/or genome sequencing (GS). Information regarding methodological approach, costs, effects and time horizon was abstracted from these publications. Twenty-eight economic evaluations of ES/GS within pediatrics were identified. Costs included were mainly restricted to direct in-hospital healthcare costs and varied widely in inclusion of sort of costs and time horizon. Nineteen studies included diagnostic yield and eight studies included cost-effectiveness as outcome measure. Studies varied greatly in terms of included sort of costs data, effects and time horizon. Large differences in inclusion of cost and effect parameters were identified between studies, validity of outcomes can therefore be questioned and it hinders valid comparison and wide-spread generalization of conclusions. In addition to current health economic guidance, specific guidance for evaluations in pediatric care is therefore necessary to improve validity of outcomes and furthermore facilitate comparable decision-making for implementing novel NGS-based diagnostic modalities in pediatric genetics and beyond.

2.1 INTRODUCTION

Worldwide, approximately 350 million people are affected by rare disorders of which fifty percent had an onset during childhood, and eighty percent has a genetic origin [1, 2]. The diagnosis of rare genetic disorders in children is challenging and time consuming due to among others the rarity of the individual disorder, the variability of the clinical manifestations, the genetic heterogeneity and the deficiencies in laboratory testing. Recent developments in Next Generation Sequencing (NGS) have made it possible to investigate all protein-coding regions or even entire genome in one single time, i.e. by implementing exome sequencing (ES) or genome sequencing (GS), respectively [3]. These developments have resulted in an increase in genetic diagnoses and a shortened time-to-diagnosis for patients with expected genetic disorders [4, 5].

These rapid technological developments are often of clinical relevance, and subsequently studies on economic impact are increasingly being performed to assess the (added) value of new diagnostic modalities. To ensure valid decision-making and high-quality studies, it is essential that these studies adhere to respective health economic guidelines. Unfortunately, a recent study has indicated that there currently is a lack of adherence to these “basic health economic” guidelines in economic evaluations of pediatric genetics [6].

Unfortunately, adherence to these “basic health economic” guidelines does not hundred percent ensure the validity and quality of evaluations. Recent studies in other areas have indicated the need for additional, so-called disease-specific guidance, in order to ensure right choices are made on a disease level with regard to inclusion of cost data, outcome parameters and length of the analysis [6]. In addition to adherence to existing guidance it is therefore also essential to adhere and or to include (newly) developed guidance published in scientific journals, guidance which increases disease specific uniformity of methods and outcome measures. This would not only facilitate the ability to share and combine health economic data, but would possibly also lead to a decrease in research waste. An excellent example of such disease-specific guidance is the publication of Buchanan in which they outline characteristics which should be included in economic evaluations [7].

Moreover, next to a complete and comparable collection of costs and adherence to existing guidelines, there is ongoing discussion about the inclusion of effect measures for genetic technologies. In the field of health economics, combining costs and effects into one outcome measure, such as cost per life year gained or cost per quality adjusted life year gained, is routinely used and subsequently decision makers to decide on reimbursement [8]. However, using a uniform effect measure, i.e. an outcome

measurement which makes it easier to compare or combine studies, is very challenging within the field of pediatric genetics, as most often, obtaining a genetic diagnosis, does not immediately lead to treatment options.

Although recent reviews have indicated that challenges with comparability are prevailing in economic evaluations of pediatric genetics, they did not outline whether differences in included costs and effects are prevalent and which costs and effects were included by the individual studies [6, 9]. Also, assessment on inclusion of disease-specific recommendations is often lacking.

An overview of all parameters used so far within previous economic evaluations is currently lacking. We therefore aim to review all economic evaluations of ES and/or GS performed for pediatric onset genetic disorders, identifying all costs, effects and time horizon (i.e. time over which data was collected) included, and providing the differences in approaches taken. Approaches are compared to disease-specific guidance as recently recommended [7, 10].

2.2 METHODS

Study design and search strategy

A systematic literature review was conducted (February 14, 2021) to identify published economic evaluations, in which costs and effects are compared (model-based, prospective and retrospective) of the clinical applications of ES and/or GS as diagnostic tool for rare genetic disorders in a pediatric setting. Hereto, the following search strategy was applied: (sequence analysis OR high throughput nucleotide sequencing OR next generation sequencing OR whole genome sequencing OR whole exome sequencing) AND (costs and cost analysis OR cost-effectiveness) AND (children OR infant OR pediatric OR paediatric). The databases used for the search were PubMed/MEDLINE and EMBASE. Two independent reviewers validated the published articles based on the following inclusion criteria: (i) the full-version of the article was available; (ii) the article was published in English; (iii) ES and/or GS are part of the economic evaluation described; and (iv) the study population consists of children aged 18 years or younger. The independent reviewers participated in both data extraction and article screening. Both reviewers independently selected studies suitable for data analysis based on the inclusion criteria. Discrepancies were resolved by discussing the article in question. There were no restrictions considering year of publication, since the role of NGS in genetic diagnostics (ES and/or GS) has only became more evident since 2009. Of all included studies, reference lists were reviewed to identify additional studies.

Data extraction

Characteristics including study population, health condition, sample size, comparison of genetic tests, time horizon, type of included costs (for example costs related to diagnostic testing or non-medical costs) and effects (i.e. outcome measures) were extracted and summarized to create an overview of all economic evaluations included in this study. Time horizon was defined as the duration over which costs and effects were included. For each included study, the final conclusion was extracted.

Data analysis

The included costs were compared to the cost components as stated by Drummond et al. [10] and Buchanan et al. [7] to determine which aspects are currently missing in the evaluations. Drummond et al. [10] outlined that the following costs should be included: (i) costs within the healthcare sector, consisting of all medical costs directly resulting from the intervention and costs incurred during life years gained; (ii) costs for the patient and family, for example travel expenses, own contributions, time spending costs or costs of informal care; and (iii) costs in other sectors, which can be costs incurred in sectors outside the healthcare system, for municipal services, education or voluntary work.
Buchanan et al. [7] defined eight cost components, which were specifically attributable to the evaluation of genomic technologies: costs related to: (i) patient recruitment (e.g. publicity and education of patients); (ii) blood or tissue sample collection; (iii) sample testing; (iv) data analysis; (v) communication of test results; (vi) actions taken based on tests results; (vii) training and infrastructure (e.g. costs related to staff training); and (viii) indirect costs. In order to judge which effects should be measured during an economic evaluation, an overview of all included effects was created.

2.3 RESULTS

Studies identified

Based on our database searches, we identified 313 studies in Pubmed/MEDLINE and 494 studies in EMBASE, resulting in 807 unique studies. Of these, 28 studies (3.5%) fulfilled our inclusion criteria. Based on the PRISMA reporting guidelines [11], an overview of the complete selection procedure is shown in Figure 1. Manual inspection of the reference lists from these twenty-eight studies did not yield any new studies.

In Table 1, the characteristics of the included studies are summarized. The rare disorders of the children for which the child received ES/GS (i.e. intellectual disability, epilepsy, autism spectrum disorder) and the cohort sizes varied (IQR 40-300, median 101). Nine studies (32.1%) included a scenario analysis [12-20] and six (21.4%) investigated the implementation of GS instead of, or in addition to, ES [15, 17, 21-24].

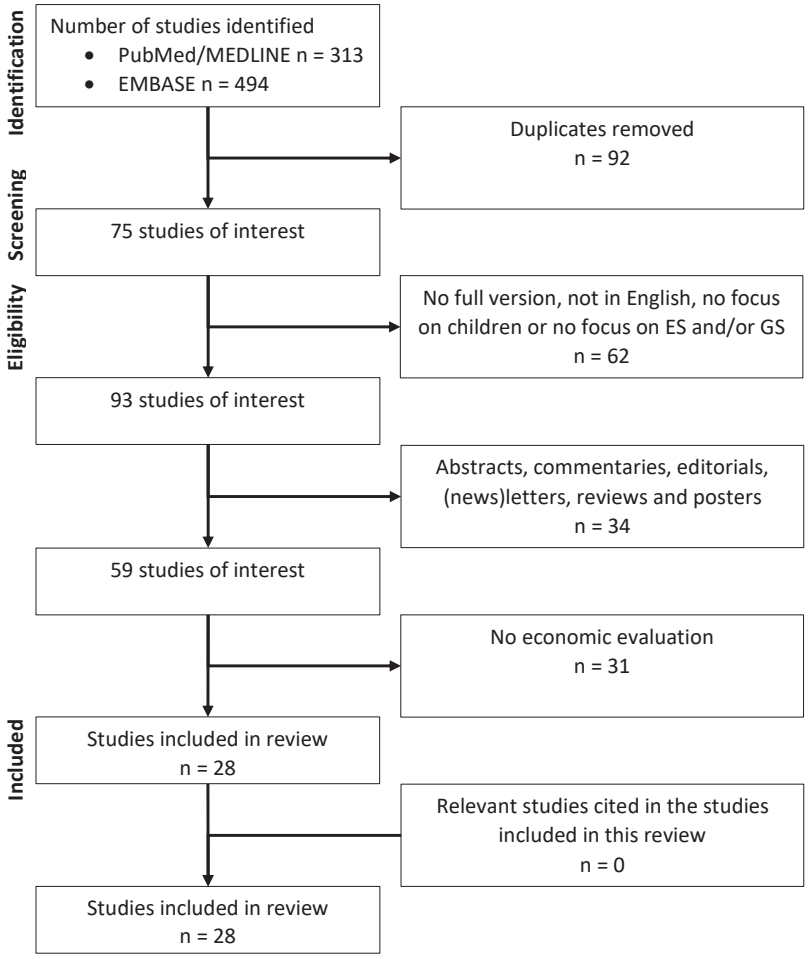


Figure 1. Overview of systematic database search.
ES = exome sequencing; GS = genome sequencing.

Time horizons were also different for the included studies, and there was no consensus regarding the moment a study started nor the moment a study ended (Table 1). In more detail, five studies (17.9%) had a time horizon of a fixed duration (range 7-24 months) [14, 15, 22, 26-27] and six studies (21.4%) included the complete diagnostic trajectory [16, 19-20, 23, 28-29]. There were eight studies (30.8%) which started at a certain moment in time (i.e. first visit in hospital, onset of symptoms or moment of inclusion) until a diagnosis was found [12, 18, 30-35]. Five studies (17.9%) looked at what had happened after ES was performed [17, 21, 24-25, 36] and the remaining four studies (15.4%) included a time period from onset of symptoms or first visit to the hospital until ES was initiated [13, 37-39]. The final conclusions of the included studies are shown in Table 1.

Table 1. Overview of characteristics of studies included in this review

Study	Country	Health condition	Total sample size	Genetic test	Time horizon	Conclusion
Soden et al. [22]	United States	NDD	119	Standard ES versus rapid GS	First 33 months of diagnostic trajectory	Sequencing exomes or genomes should become an early part of the diagnostic workup for NDD
van Nimwegen et al. [12]	Netherlands	Neurological disorders	50	Standard diagnostic trajectory versus: 1) ES, by which ES replaces: • All genetic tests currently used 2) ES, by which ES replaces: • All genetic tests • Repeated and burdensome tests • 50% of the physician visits	From first physician visit in the university hospital until a diagnosis was found	Novel diagnostic strategies, including NGS, should be evaluated
Valencia et al. [37]	United States	Genetic disorders	40	Standard ES in addition to or replacing standard diagnostic trajectory	All diagnostic tests performed before ES	Implementing ES is clinically useful and cost-effective
Joshi et al. [30]	United States	Early onset epileptic encephalopathies	4	ES versus standard diagnostic trajectory	From inclusion until diagnosis was found	Cost savings if ES was performed early in diagnostic trajectory
Sabatini et al. [19]	United States	Non-small cell lung cancer, sensorineural hearing loss, neurodevelopmental disorders	-	Three different diagnostic trajectories including ES	Complete diagnostic trajectory	Both sequencing technology and cost savings will improve over time, so cost-impact will be refined in the future
Nolan et al.[38]	United States	Pediatric neurological disorders	53	ES versus secondary genetic and metabolic testing	All diagnostic tests performed before ES	ES could allow for more efficient and fruitful diagnostic neurological evaluations

Table 1. Continued.

Study	Country	Health condition	Total sample size	Genetic test	Time horizon	Conclusion
Monroe et al. [13]	Netherlands	Intellectual disability	17	ES versus standard diagnostic trajectory: • Diagnosed patients: ES replaces all genetic costs (except aCGH and SNP arrays) and metabolic assessments • Undiagnosed patients: ES replaces all genetic costs (except aCGH and SNP arrays) • Both groups: assumption that ES results in cost reduction of 50% for health-care visits, imaging, biochemical investigations and patient day admission	From first visit to hospital until initiation of ES	Early implementation of ES is relevant and cost-effective
Schofield et al. [31]	Australia	Muscle diseases	56	Standard diagnostic trajectory versus molecular diagnostic trajectory (Neuromuscular gene panel or ES)	From referral of patients with suspicion of a diagnosis until genetic diagnosis or return of ES if no genetic diagnosis was found	Cost-effectiveness of implementing ES increases data reanalysis, testing first-degree relatives and parental reproductive outcomes are taken into account
Vissers et al. [33]	Netherlands	Neurological disorders of suspected genetic origin	150	ES versus standard diagnostic trajectory	From first visit to hospital clinic until diagnosis was established	ES results in a higher diagnostic yield without increasing the costs
Hayeems et al. [21]	Canada	Developmental delay	101	GS versus CMA	All clinical care activities prompted by CMA or GS	The clinical and economic consequences of implementing GS depend on the evaluation of downstream care and cost consequences

Table 1. Continued.

Study	Country	Health condition	Total sample size	Genetic test	Time horizon	Conclusion
Stark et al. [32]	Australia	Childhood syndromes	40	ES versus standard diagnostic trajectory, by implementing ES three different moments in time: 1) ES after basic & complex investigations 2) ES after basic investigations 3) ES immediately after admission	From age of onset symptoms until a diagnosis was established or an uninformative ES report was issued	Implementing ES early in the diagnostic trajectory significantly increases diagnostic rate and decreases cost per diagnosis
Tan et al. [14]	Australia	Suspected monogenic disorders	40	ES versus standard diagnostic trajectory, by comparing four pathways: 1) Standard diagnostic trajectory without ES 2) Standard diagnostic trajectory with ES 3) ES applied at first clinical genetics assessment 4) ES at tertiary presentation	Children suspected of having a monogenic condition were followed for seven months	ES is associated with a higher diagnostic yield and cost-effectiveness is maximized by implementing ES early in the diagnostic trajectory
Tsiplova et al. [15]	Canada	Autism spectrum disorder	300	Three models, comparing: • CMA and ES versus CMA • CMA versus GS • CMA and ES versus GS	Fixed time period of five years	The added value of ES and GS will increase over time, since costs will decrease and clinical benefit will increase
Dillon et al. [16]	Australia	Suspected monogenic disorders	145	ES versus three disease-specific panels recommended by clinical experts	Complete diagnostic trajectory	ES has a broader coverage with no change in diagnostic costs
Yuen et al. [17]	Canada	Autism spectrum disorder	1000	Four models, comparing: • CMA only • CMA and ES • ES only • GS only	Two years, starting from time of autism spectrum disorder diagnosis	At the moment, ES or GS does not lead to cost savings, but can become cost-effective. Focus should not only be on diagnostic yield, but also on utility and the increasing need for genetic services

Table 1. Continued.

Study	Country	Health condition	Total sample size	Genetic test	Time horizon	Conclusion
Stark et al. [25]	Australia	Rare diseases	80	Re-analysis of ES results compared with ongoing standard diagnostic testing	From ES disclosure (February 2014) until the end of study (October 2016) with a minimum follow-up of twelve months	This study supports early implementation of ES
Howell et al. [18]	Australia	Epilepsy	114	ES at different stages during diagnostic tract	From epilepsy onset until diagnosis found	Early implementation of ES leads to increase in diagnostic yield associated with lower costs
Vrijenhoek et al. [26]	Netherlands	Intellectual disability	370	ES at different stages during diagnostic tract	From first visit to hospital until a fixed moment in time	Implementing ES can be cost-effective
Stark et al. [34]	Australia	Pediatric care	40	Rapid ES versus standard diagnostic trajectory	From age of onset symptoms until a diagnosis was established or an uninformative ES report was issued	Rapid ES is associated with high diagnostic and clinical utility and is cost-effective
Palmer et al. [28]	Australia	Epileptic encephalopathy	32	ES versus standard diagnostic trajectory	Complete diagnostic trajectory	Use of ES is cost-effective and has a higher clinical utility
Demos et al. [35]	Canada	Early-onset epilepsy	180	ES versus standard diagnostic trajectory	From genetic counseling until research validation of the variant	Performing ES early in the diagnostic trajectory affects diagnostic yield, clinical utility and potential cost-effectiveness positively

Table 1. Continued.

Study	Country	Health condition	Total sample size	Genetic test	Time horizon	Conclusion
Radio et al. [39]	Italy	Rare diseases	324	ES versus standard diagnostic trajectory	Onset of symptoms until a ES was performed	ES decreases the diagnostic trajectory and positively influences management of undiagnosed patients
Schofield et al. [36]	Australia	Suspected monogenic disorders	80	ES versus standard diagnostic trajectory, by implementing after basic investigations	From age of onset symptoms until 473 days post result	Use of ES is cost-effective
Yokoi et al. [29]	Japan	Multiple congenital anomalies and intellectual disability	200	ES versus aCGH	Complete diagnostic trajectory	ES saves costs and time
Dragojlovic et al. [23]	Canada	Suspected genetic disorders	498	Standard diagnostic trajectory including GS versus standard diagnostic trajectory without GS	Complete diagnostic trajectory until GS was performed	For economic models, longer time horizons need to be included. Especially for patients without a conclusive diagnosis, long-term costs may influence conclusions about cost-effectiveness of GS
Kosaki et al. [20]	Japan	Suspected monogenic disorders	360	ES at different stages during diagnostic trajectory	Complete diagnostic trajectory	ES as first-tier test leads to cost-savings, but more research is needed to see whether this also applies to other countries
Smith et al. [27]	United States	Suspected genetic disorders	368	ES versus standard diagnostic trajectory	One year, starting from the first genetics consult	More research is needed in order to define the most optimal use of ES
Yeung et al. [24]	Australia	Complex, suspected monogenic disorders	92	GS versus standard diagnostic trajectory	Fixed time period of three years after diagnosis	GS leads to increased diagnostic yield and decrease in costs

NDD = neurodevelopmental disorders; ES = exome sequencing; GS = genome sequencing; aCGH = microarray-based comparative genomic hybridization; SNP = single nucleotide polymorphism; CMA = Chromosomal microarray; NGS = next-generation sequencing

Included costs and effects

Table 2 shows a summary of the different cost categories according to both Drummond et al. [10] and Buchanan et al. [7]. Regarding inclusion of internal costs (i.e. diagnostic and non-medical costs within the hospital of interest), external costs (i.e. costs outside or in another hospital) and additional costs, results are diverse: fourteen studies (50.0%) included only internal costs [13, 15-16, 20, 22, 24, 26-30, 35, 37, 39], whereas eleven studies (42.3%) also took a part of the external costs into account [12, 18-19, 21, 25, 31-34, 36, 38]; two studies (7.7%) also investigated nonmedical costs like travelling costs [14, 23] and time spending costs for (parents of) the patient, such as time lost due to medical visits [17, 23]. Furthermore, eleven studies (42.3%) solely focused on costs of diagnostic testing [15-16, 19, 22, 29, 31-33, 35, 37-38]. None of the studies took costs incurred during life years gained or costs related to other sectors (non-medical costs) into account.

Compared to the cost components of Buchanan et al. [7], all of the studies included costs related to sample collection, sample testing and data analysis. Fourteen out of twenty-eight studies (50.0%) took post-test counseling into account [12-14, 17, 20-21, 23-28, 30, 39] and nine out of twenty-eight studies (32.1%) took action taken based on test result into account [12-15, 21, 24-26, 30]. Three studies (11.5%) included non-medical costs, such as travel expenses and time spending costs of patients and family [14, 17, 23]. Costs related to patient recruitment and training and infrastructure were not investigated.

Table 3 shows a summary of the investigated effects. Nineteen studies (67.9%) included diagnostic yield [12-14, 16, 20, 22, 24-29, 31-33, 35-38]. Two studies (7.7%) took the number of ongoing pregnancies and utilization of reproductive genetic services into account [25, 36]. Considering cost-related outcomes, included outcome effects varied: five studies (19.2%) focused on cost-effectiveness only [14, 22, 31-32, 37], but the more recent publications included incremental costs per additional positive finding in (hypothetical) testing scenarios [15-16], incremental costs per additional diagnosis [17-18, 25, 28] or an incremental cost-effectiveness ratio (ICER) [36].

Table 2. Included costs categories according to Drummond et al. [10] and Buchanan et al. [7]

	Soden et al. [22]	Nimwegen et al. [12]	Valencia et al. [37]	Joshi et al. [30]	Sabatini et al. [19]	Nolan et al. [38]	Monroe et al. [13]	Schofield et al. [31]	Vissers et al. [33]	Hayeems et al. [21]	Stark et al. [32]	Tan et al. [14]	Tsiplova et al. [15]	Dillon et al. [16]	Yuen et al. [17]	Stark et al. [25]	Howell et al. [18]	Vrijenhoek et al. [26]	Stark et al. [34]	Palmer et al. [28]	Demos et al. [35]	Radio et al. [39]	Schofield et al. [36]	Yokoi et al. [29]	Dragojlovic et al. [23]	Kosaki et al. [20]	Smith et al. [27]	Yueng et al. [24]
Included cost categories according to Drummond et al. [10]																												
Costs within the healthcare sector																												
- Costs incurred during life years gained																												
Internal costs																												
- Costs genetic diagnostics	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
- Costs other diagnostics	X	X		X		X	X	X	X	X	X	X			X	X	X	X	X	X	X	X		X	X	X	X	X
- Other medical costs		X		X			X			X		X			X	X	X	X	X	X		X	X		X	X	X	X
External costs																												
- Diagnostic costs in different hospitals		X			X	X		X	X	X	X					X	X		X				X				
- Other medical costs in different hospitals		X								X						X								X				
Costs for patient and family																												
- Travel expenses												X												X				
- Own contributions/co-payments																												
- Time spending costs															X									X				
- Costs of informal care																												
Costs in other sectors																												
- Non-medical costs (e.g. municipal services, education or voluntary work)																												

Table 2. Continued.

	Soden et al. [22]	Nimwegen et al. [12]	Valencia et al. [37]	Joshi et al. [30]	Sabatini et al. [19]	Nolan et al. [38]	Monroe et al. [13]	Schofield et al. [31]	Vissers et al. [33]	Hayeems et al. [21]	Stark et al. [32]	Tan et al. [14]	Tsiplova et al. [15]	Dillon et al. [16]	Yuen et al. [17]	Stark et al. [25]	Howell et al. [18]	Vrijenhoek et al. [26]	Stark et al. [34]	Palmer et al. [28]	Demos et al. [35]	Radio et al. [39]	Schofield et al. [36]	Yokoi et al. [29]	Dragojlovic et al. [23]	Kosaki et al. [20]	Smith et al. [27]	Yueng et al. [24]
Included cost categories according to Buchanan et al. [7]																												
Patient recruitment																												
Sample collection	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Sample testing	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Data analysis	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Communication of test results		X		X			X			X		X			X	X		X		X		X			X	X	X	X
Action taken based on test results		X		X			X			X		X	X			X		X										X
Training and infrastructure																												
Indirect costs												X			X										X			

Table 3. Effect measures of the studies included in this review

Year of publication	2014	2015		2016				2017						2018							2019			2020			
Study	Soden et al. [22]	Nimwegen et al. [12]	Valencia et al. [37]	Joshi et al. [30]	Sabatini et al. [19]	Nolan et al. [38]	Monroe et al. [13]	Schofield et al. [31]	Vissers et al. [33]	Hayeems et al. [21]	Stark et al. [32]	Tan et al. [14]	Tsiplova et al. [15]	Dillon et al. [16]	Yuen et al. [17]	Stark et al. [25]	Howell et al. [18]	Vrijenhoek et al. [26]	Stark et al. [34]	Palmer et al. [28]	Demos et al. [35]	Radio et al. [39]	Schofield et al. [36]	Yokoi et al. [29]	Dragojlovic et al. [23]	Kosaki et al. [20]	Smith et al. [27]	Yueng et al. [24]
Clinical effect measures																											
Clinical-effectiveness	X																										
Diagnostic yield	X	X	X			X	X	X	X		X	X		X		X		X		X	X		X	X		X	X	X
Diagnostic sensitivity	X																											
Duration of diagnostic trajectory		X																	X		X		X	X	X			
Diagnostic rate						X																				X		
Healthcare consequences										X																		X
Number of diagnoses															X		X											
Number of ongoing pregnancies																X							X					
Number of reproductive genetic services used																X							X					
Differences in short term resource use and wait time between genetic testing strategies															X													
Survival																										X		
Economic effect measures																												
Cost-effectiveness	X		X					X			X	X				X				X			X					
Cost savings				X		X	X		X							X		X	X	X	X		X			X		X
Cost impact					X					X																			
Cost ranges					X																	X						
Healthcare resource use		X														X												
Associated direct medical costs		X																										

Table 3. Continued.

Year of publication	2014	2015		2016				2017						2018								2019			2020				
Study	Soden et al. [22]	Nimwegen et al. [12]	Valencia et al. [37]	Joshi et al. [30]	Sabatini et al. [19]	Nolan et al. [38]	Monroe et al. [13]	Schofield et al. [31]	Vissers et al. [33]	Hayeems et al. [21]	Stark et al. [32]	Tan et al. [14]	Tsiplova et al. [15]	Dillon et al. [16]	Yuen et al. [17]	Stark et al. [25]	Howell et al. [18]	Vrijenhoek et al. [26]	Stark et al. [34]	Palmer et al. [28]	Demos et al. [35]	Radio et al. [39]	Schofield et al. [36]	Yokoi et al. [29]	Dragojlovic et al. [23]	Kosaki et al. [20]	Smith et al. [27]	Yueng et al. [24]
Costs in general													X	X			X	X						X	X	X	X	X
Incremental costs per additional positive finding in (hypothetical) testing scenario's													X	X														
Cost per diagnosis																X	X		X	X			X	X	X		X	
Cost per patient															X	X	X		X	X	X	X	X					X
QALY																X							X					
Cost of reanalysis																X							X					
Incremental cost per additional diagnosis															X	X	X			X								
Costs after diagnosis																		X										
Yearly costs of diagnostic trajectory																								X				
Total and yearly cost of diagnostic delay																						X						
ICER																							X					
Costs of parental reproductive costs																							X					

QALY = Quality-adjusted life year; ICER = Incremental cost effectiveness ratio

2.4 DISCUSSION

Based on our inclusion criteria, to date twenty-eight economic evaluations regarding NGS have been performed within the pediatric population. These economic evaluations were published between 2014 and 2020. Our review outlined the presence of an extensive variability in choice and usage of costs, effects and time horizon in these economic evaluations. According to the cost categories defined by Drummond et al. [10], costs included by the studies mainly focused on diagnostic costs. Only three out of twenty-eight took other healthcare resources (i.e. travel expenses [14, 23] and time spending costs [17, 23]) into account, while personal costs (i.e. costs of informal care, own contributions/co-payments and non-medical costs) were not included at all. The cost categories as defined by Drummond [10] and Buchanan et al. [7] focus on the costs related to (genetic) diagnostics and beyond diagnostics. However, no uniformity can be found between the cost taken into account by the different studies in this review. In order to increase uniformity, future studies should at minimum follow their guidelines [7, 10]. Since it will be challenging to include non-medical costs, we suggest to also include at least non-diagnostic costs in addition to the diagnostic costs. If studies work conform to this approach, this would also improve insight in and appreciation of cost-effectiveness of ES and GS [23]. This is also supported by a study of Vrijenhoek et al. [26], which stated that benefit of genetic diagnostics can be broader than the diagnostic test itself. Having a diagnosis can also influence future health, treatment can be started earlier or expensive surgeries can be prevented.

Considering time horizon, no uniform method was used to determine the start and end of a study. Most of the studies ended the economic evaluation when a final diagnosis was found or when ES was initiated. However, the total duration included by the studies varied widely. This confirms the need for a uniform approach to ensure comparability. A study of Dragojlovic et al. [23] concluded that longer time horizons need to be included. Especially in patients without a conclusive diagnosis, long-term costs may influence conclusions about cost-effectiveness of a diagnostic test. It is not possible to judge whether studies which took a fixed time-period as time horizon also took the complete diagnostic trajectory into account. To capture the full benefit of ES and/or GS, at least the complete diagnostic trajectory should be taken into account. According to Dragojlovic et al. [23], a longer follow-up period of at least two or three years is needed to capture the health benefit following having or not having a diagnosis. The latter may even suggest that stratification according to the outcome of the genetic test should be performed, as differences may be expected.

Interestingly, there seems to be a shift in focus on how effects were studied over time. The first publications regarding evaluations of NGS in pediatric genetics include

effects directly related to the diagnostic trajectory, for instance diagnostic yield or cost-effectiveness itself. In health economic evaluations, a quality-adjusted life year (QALY) is most often used as effect measure to calculate the incremental cost effectiveness ratio (ICER) [40]. Since the end of 2017, it is common to use the ICER used to investigate cost-effectiveness within the field of health technology assessment. Although Stark et al. [25] did investigate QALYs, this was not included in an ICER. The first two studies which calculated an ICER, included additional positive findings in scenario analysis as effect measure [15-16]. Later on, effects were replaced by additional number of diagnoses [17-18]. A more recent study of Schofield et al. [36] included QALYs to calculate the ICER. Although valuable, making decisions on willingness to pay per additional diagnoses is difficult. Besides, also no conclusive diagnosis can have added value to both doctors and (parents of the) patients. For example, in case of a severe disease. Expensive care might be continued in case no severe diagnosis can be found. For decision makers, QALYs are very well-known parameters in de the decision-making process. Other outcome measures, such as clinical utility, are more difficult to base reimbursement decisions upon, although these better reflect the added clinical value within the pediatric population. The findings of this study again demonstrated that more discussions should be initiated with all decision makers to gain complete insight in most valuable other outcome measures compared to the QALY to base a decision upon.

Remarkably, two studies included the number of ongoing pregnancies and the utilization of parents' reproductive genetic services as effect measure [25, 36]. This result also suggests that including costs goes beyond including costs directly related to the patient (child) him/herself. That is, in daily practice, guidance based on genetic test results is often not limited to impacting (future) life decisions of the patient him/herself, but also affects the choices and health care costs of his/her (blood)relatives. In order to perform economic evaluations in NGS, it is important to include all relevant information. However, at the moment it is unclear how this relevant information can be defined and to what extent it is needed to involve effect measures related to the relatives of the child. These findings confirm the need of a uniform approach, including all cost aspects which should be the minimum requirement to guide decision-making.

Recently, it has been outlined that non-adherence to current health economic checklists is a major issue in economic evaluations of NGS for pediatric patients [6]. Although Alam and Schofield [6] make a valid point regarding non-adherence, they did not discuss that disease-specific guidance to perform an economic evaluation in a certain setting was lacking. Examples include for instance that the use of terminology by the included papers was very divers (Table 3), but also that is was unclear what was meant by the terms in absence of definitions, making comparisons and interpretation difficult. Whereas these at large may depend on the study perspective, also these were not clearly outlined.

The end results are economic evaluations within pediatrics for which it is underdefined which costs to include or how to define these. Such practical, disease-specific guidance is needed to ensure the improvement of future economic evaluations in genetics and thereby ensure the correct collection of highly valid data which can be used by the entire scientific field. These improvements can be created by more guidance as many authors of economic evaluations take previous papers as example when deciding upon inclusion of costs; high quality standards should therefore be developed and also journals should be made aware of the importance of so called cross-validation.

It was unclear whether or not studies had the intention to perform a full economic evaluation. In order to create more uniformity and comparable studies, it is essential that studies indicate whether the objective was to conduct a full-economic evaluation and or whether there was a transparent reason to include certain costs and or effects in the evaluation.

The variability in choice of costs and effects also indicates the lack of discussion and guidance with current decision makers within the field. It is highly relevant that such a discussion takes place to ensure inclusion of the most valuable outcome measures for making decisions on reimbursement and timing of expensive diagnostics. Within pediatric genetics, a disease specific guidance as outlined in this review is needed. This guidance would increase quality, reliability and comparability of outcomes. Current initiatives such as the GEECS (Global Economics and Evaluation of Clinical Genomics Sequencing Working Group) are essential in this case [41]. Within GEECS, for instance, they focus on improving methods used for assessing value of new genomic technologies. The lack of uniformity and consensus on outcome measures (as indicated in this study) indicates that guidance and consensus on outcome measures should have high priority to ensure and improve decision-making.

At last, the majority of the included evaluations in recent reviews have had a focus on the implementation of ES [12-14, 16, 18-20, 25-39], and only six studies investigated the implementation of GS [15, 17, 21-24]. In order to help decision makers with future decisions, especially in a fast-developing field such as medical genetics, it would be very relevant to outline which outcomes and individual input parameters are useful for future decisions on for instance implementation of GS in clinical practice. If achieved, this will also improve the power of evidence and, better guide future research on cost-effectiveness studies towards those areas for which evidence is still largely unexplored. Within the field of medical genetics, much effort is being made to share knowledge on genetic causes of disease, similar efforts should be made to push the field of economic analysis in genetics. Hereto, transparency in sharing outcomes and knowledge with regard to health economic evaluation are essential.

In general, it is challenging to use current health economic methods to capture the full benefit of NGS-related diagnostics. For instance, the inclusion of secondary findings, non-health benefits and family spillover effects are difficult to incorporate in cost savings, let alone in one overarching number informing decision makers. New approaches and methods should become available to fully capture, address and evaluate the added benefit. Methodological research is essential in order to more precisely estimate the impact of genetic diagnostics and to improve well informed/valid decisions in the near future.

This study has two (minor) limitations. First of all, only Pubmed/MEDLINE and EMBASE were included for database search. However, we have also manually inspected the reference lists of the included studies to ensure that all relevant studies are included in this review. This did not lead to any new inclusions. Another possible limitation of this study was that we did not perform quality checks for the included studies. Although we did use the PRISMA reporting guideline [11], no alternative quality checks were performed. However, we do not think that inclusion of extra databases or adding quality checks would result in different results and/or conclusions.

Conclusions

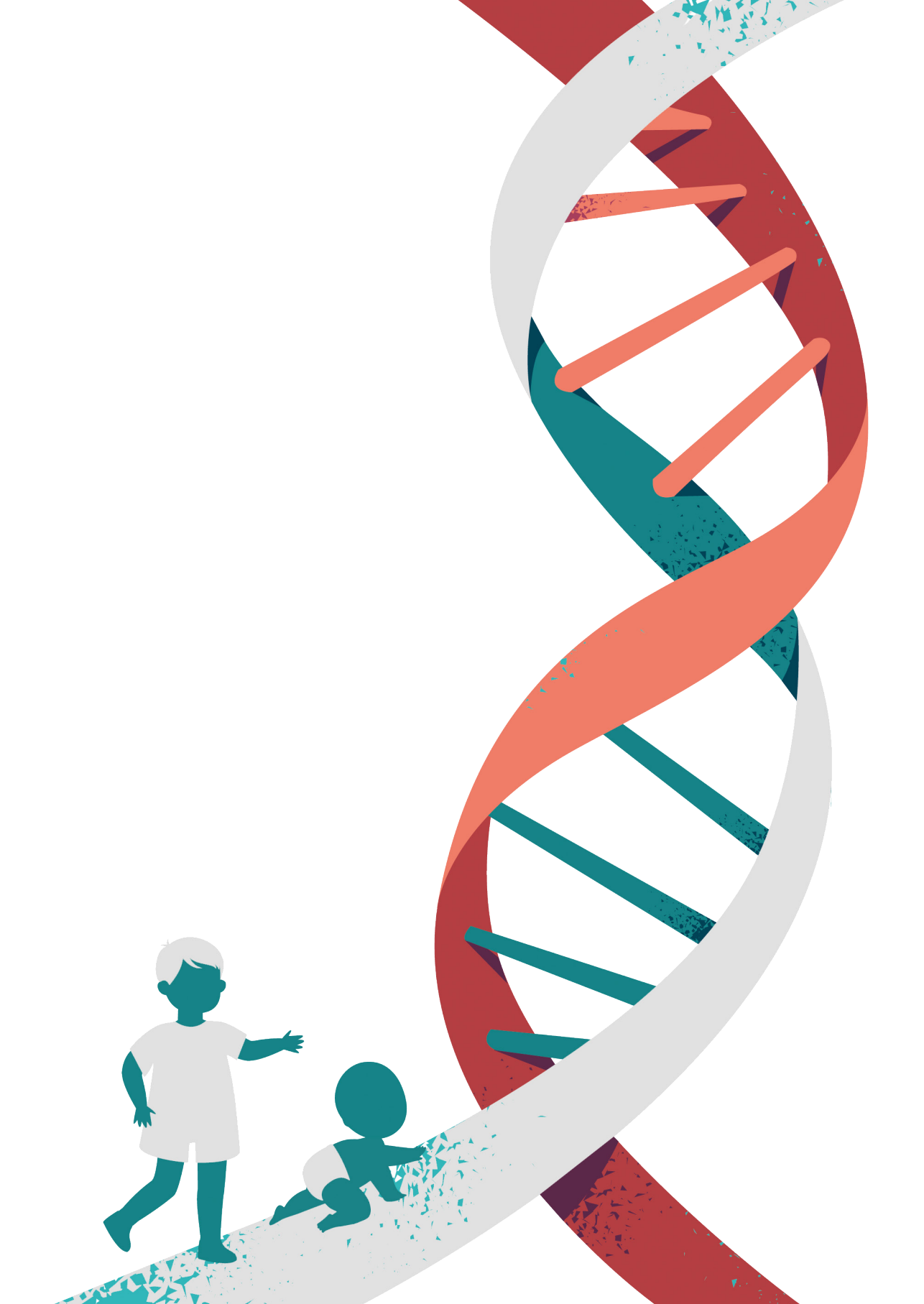
In conclusion, a large variety in choice of costing characteristics and outcome measures is present in economic evaluations of new genetic technologies in pediatric conditions. This variability shown by the included studies indicates randomness in methods for economic evaluations in NGS and hampers a reputable comparison between outcomes. We argue that an improvement in cost collection (duration and type of cost data) and standardization in outcome measure is necessary for valid decision-making in the field of pediatric genetics, and beyond. In addition to collaboration on clinical outcomes and data sharing, we should strive for a uniform approach in health economics in genetics to fight research waste but even more to speed up and improve the decision-making process; now is the time.

REFERENCES

1. Aymé, S., & Schmidtke, J. (2007). Networking for rare diseases: a necessity for Europe. Bundesgesundheitsblatt - Gesundheitsforschung - Gesundheitsschutz, 50(12), 1477–1483. https://doi.org/10.1007/s00103-007-0381-9.
2. Zurynski, Y., Frith, K., Leonard, H., & Elliott, E. J. (2008). Rare childhood diseases: how should we respond? Archives of Disease in Childhood, 93(12), 1071–1074. https://doi.org/10.1136/adc.2007.134940.
3. Bick, D., & Dimmock, D. (2011). Whole exome and whole genome sequencing. Current Opinion in Pediatrics, 23(6), 594–600. https://doi.org/10.1097/mop.0b013e32834b20ec.
4. Vrijenhoek, T., Kraaijeveld, K., Elferink, M., De Ligt, J., Kranendonk, E., Santen, G. W., Nijman, I. J., Butler, D., Claes, G. R. F., Costessi, A., Dorlijn, W., Van Eyndhoven, W., Halley, D., Van Den Hout, M. C. G. N., Van Hove, S., Johansson, L., Jongbloed, J. D. H., Kamps, R., Kockx, C., . . . Cuppen, E. (2015). Next-generation sequencing-based genome diagnostics across clinical genetics centers: implementation choices and their effects. European Journal of Human Genetics, 23(9), 1142–1150. https://doi.org/10.1038/ejhg.2014.279.
5. Koboldt, D. C., Steinberg, K. M., Larson, D. E., Wilson, R. K., & Mardis, E. R. (2013). The Next-Generation Sequencing Revolution and its impact on genomics. Cell, 155(1), 27–38. https://doi.org/10.1016/j.cell.2013.09.006.
6. Alam, K., & Schofield, D. (2018). Economic evaluation of genomic sequencing in the paediatric population: a critical review. European Journal of Human Genetics, 26(9), 1241–1247. https://doi.org/10.1038/s41431-018-0175-6.
7. Buchanan, J., Wordsworth, S., & Schuh, A. (2013). Issues surrounding the health economic evaluation of genomic technologies. Pharmacogenomics, 14(15), 1833–1847. https://doi.org/10.2217/pgs.13.183.
8. National Institute for Health and Care Excellence (NICE) [Internet] Guide to the methods of technology appraisal 2013. Available from: https://www.nice.org.uk/process/pmg9/resources/guide-to-the-methods-of-technology-appraisal-2013-pdf-2007975843781.
9. Schwarze, K., Buchanan, J., Taylor, J. C., & Wordsworth, S. (2018). Are whole-exome and whole-genome sequencing approaches cost-effective? A systematic review of the literature. Genetics in Medicine, 20(10), 1122–1130. https://doi.org/10.1038/gim.2017.247.
10. Drummond MF, Sculpher MJ, Claxton K, Stoddart GL, Torrance GW. Methods of economic evaluation in healthcare programmes. 4th ed. Oxford University Press, 2015.
11. Moher, D., Liberati, A., Tetzlaff, J., & Altman, D. G. (2009). Preferred reporting items for Systematic Reviews and Meta-Analyses: the PRISMA statement. PLOS Medicine, 6(7), e1000097. https://doi.org/10.1371/journal.pmed.1000097.
12. Van Nimwegen, K., Schieving, J., Willemsen, M., Veltman, J. A., Van Der Burg, S., Van Der Wilt, G. J., & Grutters, J. P. (2015). The diagnostic pathway in complex paediatric neurology: A cost analysis. European Journal of Paediatric Neurology, 19(2), 233–239. https://doi.org/10.1016/j.ejpn.2014.12.014.
13. Monroe, G. R., Frederix, G. W., Savelberg, S. M. C., De Vries, T. I., Duran, K., Van Der Smagt, J. J., Terhal, P. A., Van Hasselt, P. M., Kroes, H. Y., Verhoeven-Duif, N. M., Nijman, I. J., Carbo, E. C., Van Gassen, K., Knoers, N. V. a. M., Hövels, A. M., Van Haelst, M. M., Visser, G., & Van Haaften, G. (2016). Effectiveness of whole-exome sequencing and costs of the traditional diagnostic trajectory in children with intellectual disability. Genetics in Medicine, 18(9), 949–956. https://doi.org/10.1038/gim.2015.200.
14. Tan, T. Y., Dillon, O. J., Stark, Z., Schofield, D., Alam, K., Shrestha, R., Chong, B., Phelan, D., Brett, G. R., Creed, E., Jarmolowicz, A., Yap, P., Walsh, M., Downie, L., Amor, D. J., Savarirayan, R., McGillivray, G., Yeung, A., Peters, H., . . . White, S. (2017). Diagnostic impact and cost-effectiveness of Whole-Exome sequencing for ambulant children with suspected monogenic conditions. JAMA Pediatrics, 171(9), 855. https://doi.org/10.1001/jamapediatrics.2017.1755.
15. Tsiplova, K., Zur, R. M., Marshall, C. R., Stavropoulos, D. J., Pereira, S. L., Merico, D., Young, E. J., Sung, W. W. L., Scherer, S. W., & Ungar, W. J. (2017). A microcosting and cost–consequence analysis of clinical genomic testing strategies in autism spectrum disorder. Genetics in Medicine, 19(11), 1268–1275. https://doi.org/10.1038/gim.2017.47.
16. Dillon, O. J., Lunke, S., Stark, Z., Yeung, A., Thorne, N., Gaff, C., White, S., & Tan, T. Y. (2018). Exome sequencing has higher diagnostic yield compared to simulated disease-specific panels in children with suspected monogenic disorders. European Journal of Human Genetics, 26(5), 644–651. https://doi.

org/10.1038/s41431-018-0099-1.
17. Yuen, T., Carter, M. T., Szatmári, P., & Ungar, W. J. (2018). Cost-effectiveness of genome and exome sequencing in children diagnosed with autism spectrum disorder. Applied Health Economics and Health Policy, 16(4), 481–493. https://doi.org/10.1007/s40258-018-0390-x.
18. Howell, K., Eggers, S., Dalziel, K., Riseley, J. R., Mandelstam, S., Myers, C. T., McMahon, J. M., Schneider, A., Carvill, G. L., Mefford, H. C., Scheffer, I. E., & Harvey, A. S. (2018). A population-based cost-effectiveness study of early genetic testing in severe epilepsies of infancy. Epilepsia, 59(6), 1177–1187. https://doi.org/10.1111/epi.14087.
19. Sabatini, L. M., Mathews, C., Ptak, D. M., Doshi, S., Tynan, K., Hegde, M., Burke, T. R., & Bossler, A. (2016). Genomic sequencing procedure microcosting analysis and Health Economic Cost-Impact Analysis. The Journal of Molecular Diagnostics, 18(3), 319–328. https://doi.org/10.1016/j.jmoldx.2015.11.010.
20. Kosaki, R., Kubota, M., Uehara, T., Suzuki, H., Tamura, T., & Kosaki, K. (2020). Consecutive medical exome analysis at a tertiary Center: diagnostic and health-economic outcomes. American Journal of Medical Genetics- Part A, 182(7), 1601–1607. https://doi.org/10.1002/ajmg.a.61589.
21. Hayeems, R. Z., Bhawra, J., Tsiplova, K., Meyn, S., Monfared, N., Bowdin, S., Stavropoulos, D. J., Marshall, C. R., Basran, R. K., Shuman, C., Itô, S., Cohn, I., Hum, C., Gîrdea, M., Brudno, M., Cohn, R. D., Scherer, S. W., & Ungar, W. J. (2017). Care and cost consequences of pediatric whole genome sequencing compared to chromosome microarray. European Journal of Human Genetics, 25(12), 1303–1312. https://doi.org/10.1038/s41431-017-0020-3.
22. Soden, S. E., Saunders, C. J., Willig, L. K., Farrow, E., Smith, L. D., Petrikin, J. E., Lepichon, J. B., Miller, N., Thiffault, I., Dinwiddie, D. L., Twist, G. P., Noll, A., Heese, B. A., Zellmer, L., Atherton, A. M., Abdelmoity, A., Safina, N. P., Nyp, S. S., Zuccarelli, B., . . . Kingsmore, S. F. (2014). Effectiveness of exome and genome sequencing guided by acuity of illness for diagnosis of neurodevelopmental disorders. Science Translational Medicine, 6(265). https://doi.org/10.1126/scitranslmed.3010076.
23. Dragojlovic, N., Van Karnebeek, C., Ghani, A., Genereaux, D., Kim, E., Birch, P., Adam, S., Du Souich, C., Elliott, A. M., Lehman, A., Lynd, L. D., Mwenifumbo, J., Nelson, T. N., Van Karnebeek, C., Friedman, J. M., & Lynd, L. D. (2020). The cost trajectory of the diagnostic care pathway for children with suspected genetic disorders. Genetics in Medicine, 22(2), 292–300. https://doi.org/10.1038/s41436-019-0635-6.
24. Yeung, A., Tan, N. B., Tan, T. Y., Stark, Z., Brown, N. J., Hunter, M., Delatycki, M. B., Stutterd, C., Savarirayan, R., McGillivray, G., Stapleton, R., Kumble, S., Downie, L., Regan, M., Lunke, S., Chong, B., Phelan, D., Brett, G. R., Jarmolowicz, A., . . . White, S. (2020). A cost-effectiveness analysis of genomic sequencing in a prospective versus historical cohort of complex pediatric patients. Genetics in Medicine, 22(12), 1986–1993. https://doi.org/10.1038/s41436-020-0929-8.
25. Stark, Z., Schofield, D., Martyn, M., Rynehart, L., Shrestha, R., Alam, K., Lunke, S., Tan, T. Y., Gaff, C., & White, S. (2019). Does genomic sequencing early in the diagnostic trajectory make a difference? a follow-up study of clinical outcomes and cost-effectiveness. Genetics in Medicine, 21(1), 173–180. https://doi.org/10.1038/s41436-018-0006-8.
26. Vrijenhoek, T., Middelburg, E. M., Monroe, G. R., Van Gassen, K., Geenen, J. W., Hövels, A. M., Knoers, N. V. A. M., Van Amstel, H. K. P., & Frederix, G. W. (2018). Whole-exome sequencing in intellectual disability; cost before and after a diagnosis. European Journal of Human Genetics, 26(11), 1566–1571. https://doi.org/10.1038/s41431-018-0203-6.
27. Smith, H. S., Swint, J. M., Lalani, S. R., De Oliveira Otto, M. C., Yamal, J., Russell, H. V., & Lee, B. (2020). Exome sequencing compared with standard genetic tests for critically ill infants with suspected genetic conditions. Genetics in Medicine, 22(8), 1303–1310. https://doi.org/10.1038/s41436-020-0798-1.
28. Palmer, E. E., Schofield, D., Shrestha, R., Kandula, T., Macintosh, R., Lawson, J. A., Andrews, I., Sampaio, H., Johnson, A., Farrar, M. A., Cardamone, M., Mowat, D., Elakis, G., Lo, W. T., Zhu, Y., Ying, K., Morris, P., Jiang, T., Dias, K., . . . Sachdev, R. (2018). Integrating Exome sequencing into a diagnostic pathway for epileptic encephalopathy: evidence of clinical utility and cost effectiveness. Molecular Genetics & Genomic Medicine, 6(2), 186–199. https://doi.org/10.1002/mgg3.355.
29. Yokoi, T., Enomoto, Y., Tsurusaki, Y., Harada, N., Saito, T., Nagai, J., Naruto, T., & Kurosawa, K. (2020). An efficient genetic test flow for multiple congenital anomalies and intellectual disability. Pediatrics International, 62(5), 556–561. https://doi.org/10.1111/ped.14159.
30. Joshi, C., Kolbe, D. L., Mansilla, M. A., Mason, S. O., Smith, R. J., & Campbell, C. A. (2016). Reducing the cost of the diagnostic odyssey in early onset epileptic encephalopathies. BioMed Research International, 2016, 1–8. https://doi.org/10.1155/2016/6421039.

31. Schofield, D., Alam, K., Douglas, L., Shrestha, R., MacArthur, D. G., Davis, M., Laing, N. G., Clarke, N. F., Burns, J., Cooper, S. T., North, K., Sandaradura, S. A., & O'Grady, G. L. (2017). Cost-effectiveness of massively parallel sequencing for diagnosis of paediatric muscle diseases. npj Genomic Medicine, 2(1). https://doi.org/10.1038/s41525-017-0006-7.
32. Stark, Z., Schofield, D., Alam, K., Wilson, W., Mupfeki, N., Macciocca, I., Shrestha, R., White, S., & Gaff, C. (2017). Prospective comparison of the cost-effectiveness of clinical whole-exome sequencing with that of usual care overwhelmingly supports early use and reimbursement. Genetics in Medicine, 19(8), 867–874. https://doi.org/10.1038/gim.2016.221.
33. Vissers, L. E., Van Nimwegen, K., Schieving, J., Kamsteeg, E. J., Kleefstra, T., Yntema, H. G., Pfundt, R., Van Der Wilt, G. J., Brunner, H. G., Van Der Burg, S., Grutters, J. P., Veltman, J. A., & Willemsen, M. (2017). A clinical utility study of exome sequencing versus conventional genetic testing in Pediatric neurology. Genetics in Medicine, 19(9), 1055–1063. https://doi.org/10.1038/gim.2017.1.
34. Stark, Z., Lunke, S., Brett, G. R., Tan, N. B., Stapleton, R., Kumble, S., Yeung, A., Phelan, D., Chong, B., Fanjul-Fernández, M., Marum, J. E., Hunter, M., Jarmolowicz, A., Prawer, Y., Riseley, J. R., Regan, M., Elliott, J., Martyn, M., Best, S., . . . White, S. (2018). Meeting the challenges of implementing rapid genomic testing in acute pediatric care. Genetics in Medicine, 20(12), 1554-1563. https://doi.org/10.1038/gim.2018.37.
35. Demos, M., Guella, I., DeGuzman, C., McKenzie, M., Buerki, S. E., Evans, D. M., Toyota, E. B., Boelman, C., Huh, L., Datta, A., Michoulas, A., Selby, K., Björnson, B., Horváth, G., Lopez-Rangel, E., Van Karnebeek, C., Salvarinova, R., Slade, E., Eydoux, P., . . . Farrer, M. J. (2019). Diagnostic yield and treatment impact of targeted exome sequencing in Early-Onset Epilepsy. Frontiers in Neurology, 10. https://doi.org/10.3389/fneur.2019.00434.
36. Schofield, D., Rynehart, L., Shresthra, R., White, S., & Stark, Z. (2019). Long-term economic impacts of Exome sequencing for suspected monogenic disorders: diagnosis, management, and reproductive outcomes. Genetics in Medicine, 21(11), 2586–2593. https://doi.org/10.1038/s41436-019-0534-x.
37. Valencia, C. A., Husami, A., Holle, J., Johnson, J. A., Qian, Y., Mathur, A., Wei, C., Indugula, S. R., Zou, F., Meng, H., Wang, L., Li, X., Fisher, R., Tan, T., Begtrup, A., Collins, K., Wusik, K., Neilson, D., Burrow, T., . . . Zhang, K. (2015). Clinical Impact and Cost-Effectiveness of Whole Exome Sequencing as a Diagnostic Tool: A Pediatric Center's experience. Frontiers in Pediatrics, 3. https://doi.org/10.3389/fped.2015.00067.
38. Nolan, D., & Carlson, M. (2016). Whole exome sequencing in pediatric neurology patients. Journal of Child Neurology, 31(7), 887–894. https://doi.org/10.1177/0883073815627880.
39. Radio, F. C., Ruzzeddu, M., Bartuli, A., Novelli, A., Tartaglia, M., & Dallapiccola, B. (2019). Cost-effectiveness of Exome sequencing: An Italian pilot study on undiagnosed patients. New Genetics and Society, 38(3), 249–263. https://doi.org/10.1080/14636778.2019.1601008.
40. Drummond, M., Brixner, D., Gold, M. R., Kind, P., McGuire, A., & Nord, E. (2009). Toward a consensus on the QALY. Value in Health, 12, S31–S35. https://doi.org/10.1111/j.1524-4733.2009.00522.x.
41. Global Economics and Evaluation of Clinical Genomics Sequencing Working Group (GEECS). Available from: https://www.geecsecon.org/.



CHAPTER 3

Congenital anomalies and genetic disorders in neonates and infants: a single-center observational cohort study

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ABSTRACT

Neonates with genetic disorders or congenital anomalies (CA) contribute considerably to morbidity and mortality in neonatal intensive care units (NICUs). The objective of this study is to study the prevalence of genetic disorders in an academic level IV NICU. We retrospectively collected and analyzed both clinical and genetic data of all 1444 infants admitted to the NICU of the Radboudumc (October 2013 to October 2015). Data were collected until infants reached at least 2 years of age. A total of 13% (194/1444) of the patients were genetically tested, and 32% (461/1444) had a CA. A total of 37% (72/194) had a laboratory-confirmed genetic diagnosis. In 53%, the diagnosis was made post-neonatally (median age=209 days) using assays including exome sequencing. Exactly 63% (291/461) of the patients with CA, however, never received genetic testing, despite being clinically similar those who did. Genetic disorders were suspected in 13% of the cohort, but only confirmed in 5%. Most received their genetic diagnosis in the post-neonatal period. Extrapolation of the diagnostic yield suggests that up to 6% of our cohort may have remained genetically undiagnosed. Our data show the need to improve genetic care in the NICU for more inclusive, earlier, and faster genetic diagnosis to enable tailored management.

3.1 INTRODUCTION

A significant portion of neonates admitted to neonatal intensive care units are diagnosed with a genetic disorder [1]. Congenital malformations, potentially indicating an underlying genetic disorder, are estimated to be present in 13% of all admissions to neonatal intensive care units (NICUs) and remain one of the leading causes of neonatal mortality (25-34%) [2-6]. The clinical presentations of genetic disorders vary widely, from an isolated (major) congenital anomaly (CA) or multiple malformations (MCA) to more subtle clinical signs or symptoms. The diagnostic pathway is often long and requires extensive evaluations that may be invasive and costly [1]. Diagnosis of most genetic disorders in neonatal and pediatric intensive care units (NICUs and PICUs) is generally not timely enough to adequately guide acute clinical management.

Previous studies have shown that genetic disorders are a frequent cause of CA, especially MCA [1, 2]. However, the exact frequency is unknown, as percentages reported vary between 20-50%, which can mainly be attributed to cohort selection and the heterogeneity of diagnostic tools used [2, 7]. In neonates admitted to a NICU, genetic testing is generally aimed at detection of aneuploidies (such as trisomy 13, 18 and 21) or chromosomal aberrations, which in lesser extent is followed by direct testing of specific genes, guided by the patients' phenotype.

Over the last decade, novel technologies, such as exome sequencing (ES), have entered the genetic diagnostic arena. Its use in clinical settings, such as neonatal intensive care, have however been limited, as turnaround times were perceived too long (i.e. months) to impact acute or short-term clinical decision-making, and too costly compared to other genetic diagnostic testing options [8-10]. Yet, as these turnaround times and costs have decreased significantly, there is an opportunity for innovation and durable implementation of ES in the NICU setting.

To facilitate these efforts, insight into current practices, both at the level of clinical presentation as well as the uptake of (the type of) genetic testing, is essential. For this purpose, a retrospective observational study was performed in a cohort of neonates admitted to the NICU of the Radboud university medical center during a two-year period up to a post-natal age of two years.

3.2 METHODS

Retrospective cohort definitions

We collected data of all patients born between 1 October 2013 and 1 October 2015 and admitted to the level IV NICU of the Radboud university medical center. Exclusion criteria were genetic testing in the context of a known mutation within the family and/or the identification of disorders through the national neonatal blood spot screening program [11]. For the purpose of this study, we stratified the data to three different time periods, being prenatal (before birth), neonatal (day of birth – day 1, up to 28 days of life), and post-neonatal (beyond 28 days of life). In addition, patients were categorized in six groups based on the moment when a genetic disorder was suspected (prenatal, neonatal and post-neonatal period), combined with whether or not a genetic diagnosis was confirmed.

Data collection and analysis

Data was extracted from the electronic medical record (EMR) for each subject until the post-natal age of 2 years. A combination of automatic and manual data extraction was performed. Information regarding demographic data, diagnoses, and clinical geneticist consultations were manually extracted from the EMR of all patients. Genetic diagnosis was defined as a molecular, cytogenetic, or metabolic abnormality explained by a genetic disorder and related to the patient's presenting phenotype. "No genetic diagnosis" was classified as patients with (non-specific) symptoms, such as feeding difficulties or respiratory distress and physical abnormalities without confirmation of an underlying genetic disorder.

For each patient, we retrieved information on whether or not a clinical geneticist was consulted. If so, information on the date, location (inpatient /outpatient), and indication for consultation was obtained. We reviewed all genetic tests and recorded the type and result of the test, the date the specimen was received by the lab, and the date of the final report. We also included relevant tests performed prior to transfer to our institution using the information available in our EMR. Gene tests that were ordered as a panel (more than one gene), but for which results for each gene were provided separately, were entered as individual gene tests as the turnaround time may vary per gene. A conclusive diagnosis was defined as a laboratory-confirmed genetic diagnosis based on the identification of a (likely) pathogenic (class 4 and 5) variant in concordance with the patient's phenotype [12]. Of note, interpretation of variants also relies on the clinical presentation of the patient. Phenotypic presentation of (premature) neonates may differ from the presentation later in life for known genetic disorders [13]. The variants of unknown significance (VUS; class 3) were only considered clinically relevant if the phenotype matched appropriately as evaluated by expert clinical geneticists [14].

Primary end points

Primary end points were (i) confirmed genetic disorders, (ii) incidence of genetic testing, (iii) diagnostic yield of genetic testing and (iv) time-to-diagnosis (TTD). Suspicion of an underlying genetic disorder was based on the presence of one or more CA or other guiding clinical symptoms. The incidence of genetic testing was defined as the percentage of patients that received any molecular, cytogenetic or metabolic diagnostic testing. The diagnostic yield was defined as the percentage of cases for whom a conclusive molecular or cytogenetic diagnosis was identified, e.g. the identification of a class 3, 4 or 5 variant, that is compatible with the identified phenotype. The TTD was measured from the moment the first test was indicated until the return of the final conclusive genetic diagnostic report.

Identification and scoring of congenital anomalies

To identify the presence of any CA, we analyzed all EMR and scored the reported anomalies. Only anomalies that were identified prenatally or during the NICU stay were scored. CA were scored using the human phenotype ontology (HPO) terms and concomitantly grouped in 23 different organ systems [12]. CA were considered as isolated when affecting a single organ system, and as multiple in the presence of anomalies in two or more organ systems.

Statistical analysis

Normal distributed data were expressed in mean and standard deviation. Median and interquartile ranges were used in data with a skewed distribution. Statistical analysis was performed using descriptive and chi-square analyses and a two-sided Fisher's exact test for continuous variables.

3.3 RESULTS

During this 2-year timeframe, 1,470 patients were admitted to the NICU; 26 patients were excluded from the analysis, because genetic testing was performed in the context of a known familial mutation (n=22) or they were admitted after the identification of a neonatal bloodspot screening disorder (n=4) (Figure 1). This resulted in 1,444 eligible patients. The clinical characteristics of the included patients are shown in Table 1 and Supplementary Table 1.

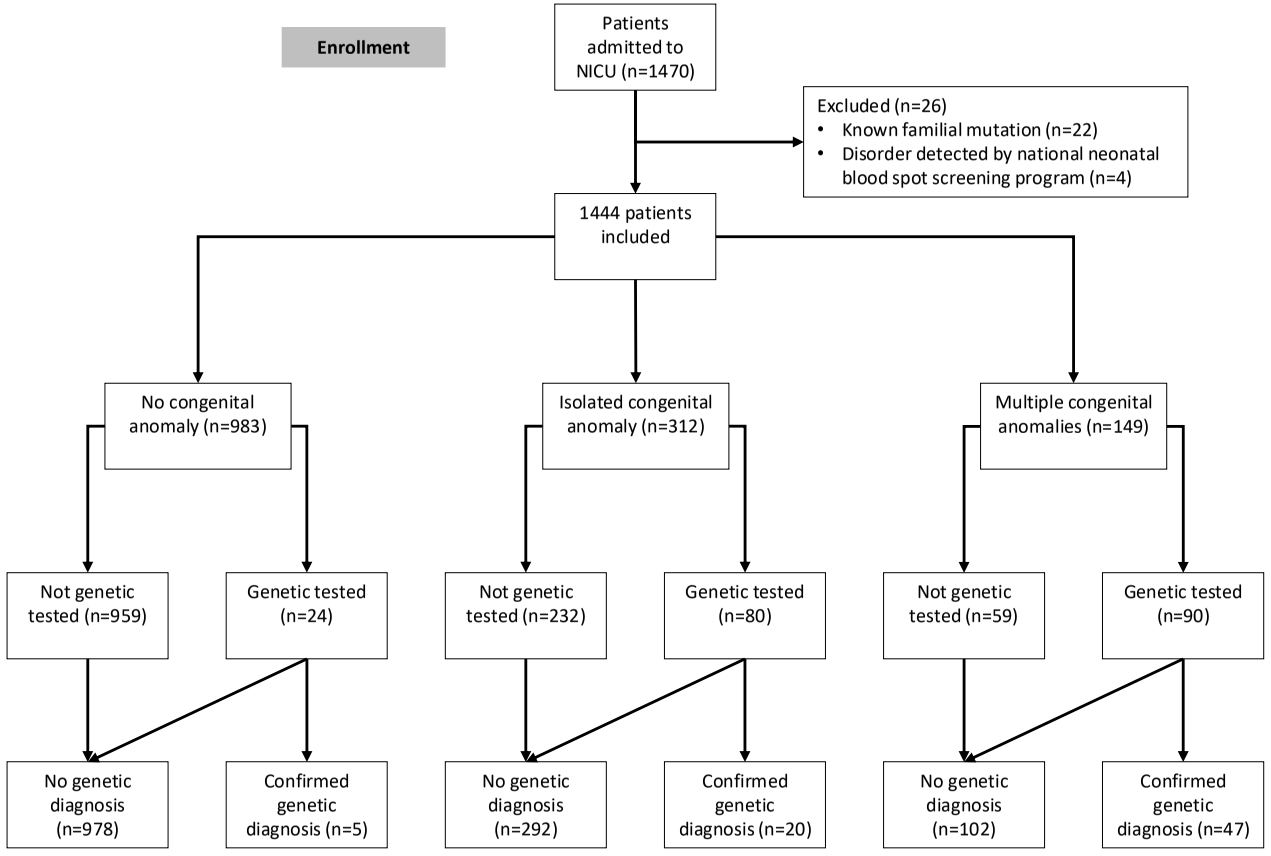

Figure 1. Flowchart of the study.
NICU = neonatal intensive care unit.

Table 1. Clinical characteristics of neonates

	N = 1,444
Male/Female	833 (58%) / 611 (42%)
Gestational age	
Extremely preterm (<28 weeks)	91 (6%)
Very preterm (28 weeks-34 weeks)	193 (13%)
Preterm (34 weeks-37 weeks	401 (28%)
Term (37 weeks-42 weeks)	746 (52%)
Post-term (>42 weeks)	9 (0.6%)
Unknown	4 (0.3%)
Congenital anomalies	
No congenital anomalies	983 (68%)
Congenital anomalies	461 (32%)
Isolated	312 (68%)
Multiple	149 (32%)
Genetic testing	
One or more genetic tests	194 (13%)

Genetic testing

In a total of 194 patients (194/1,444; 13%) 410 genetic tests were performed (Figure 2). Of these genetic tests, 28% (114/410) were ordered in the neonatal period. More than half of the genetic tests (214/410; 52%) were initiated in the post-neonatal period. The type of genetic test varied among patients and depended on the suspected genetic disorder and corresponding clinical features. In the prenatal and neonatal period quantitative fluorescent polymerase chain reaction (QF-PCR), karyotyping, and genomic microarray technologies were the most frequently used diagnostic tools, whereas in the post-neonatal period this included also Sanger sequencing and ES (Supplementary Table 2).

Genetic diagnosis

In a total of 72 patients a genetic diagnosis could be established (Table 2). The overall diagnostic yield of tested patients is 37% (72/194). We identified all genetic tests and the periods wherein these tests were performed (Supplementary Table 2). Most genetic diagnoses (38/72; 71%) were confirmed in the post-neonatal period but before 2 years of age. The timing of genetic diagnosis across the cohort is demonstrated in Tables 2 and 3. For the majority of patients receiving their genetic diagnosis in the post-neonatal period (22/38; 58%), the search for a genetic diagnosis already started in the prenatal and/or neonatal period.

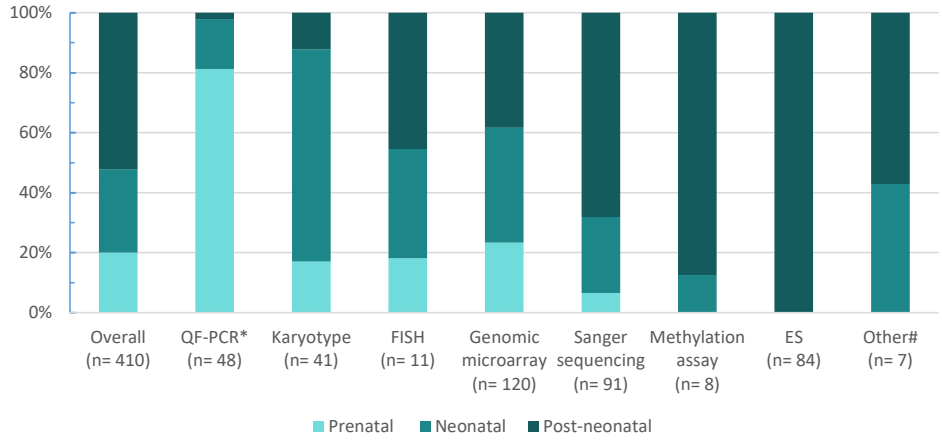

Figure 2. Percentage of all genetic tests per time period.
*Six diagnoses were confirmed with QF-PCR after abnormal noninvasive prenatal testing.; #Other: chromosome breakage and x-inactivation analysis. QF-PCR = quantitative fluorescent polymerase chain reaction; FISH = fluorescence in situ hybridization; ES = exome sequencing.

Table 2. Time period in which genetic testing started and the timing of genetically confirmed diagnosis

	Prenatally confirmed diagnosis (n=15)	Neonatally confirmed diagnosis (n=19)	Post-neonatally confirmed diagnosis (n=38)
Median time (IQR) to diagnosis (days)	N/A	22 (IQR 8)	112 (IQR 234)
Median age (IQR) start genetic testing (days)	Prenatally	2 (IQR 6)	17 (IQR 304)
Median post-natal age (IQR) at genetic diagnosis (days)	Prenatally	13 (IQR 14)	209 (IQR 483)
Median number of genetic tests	2	1	2

N/A = not applicable; IQR = interquartile range

The median TTD for patients with a confirmed genetic diagnosis in the post-neonatal period was 112 days (IQR 234 days). Patients in the post-neonatal period received more genetic tests than patients in the neonatal period (Table 3). The type of genetic tests most used in the post-neonatal period often have a long turnaround time. These factors have a significant impact on the median TTD for the patients.

Congenital anomalies

CA were identified during the NICU admission in 32% (461/1,444) of patients, of whom 68% (n=312) presented with an isolated CA and 32% with MCA (n=149; Table 1). Uptake of genetic testing correlated with the categories for CA: 24/983 (2%) of patients without CA were tested, 80/312 (26%) of patients with an isolated CA and 90/149 (60%) of

patients with MCA. As expected, also the diagnostic yield correlated with these groups, with 21% (5/24) obtained in patients without CA, 25% (20/80) for those with an isolated CA and 52% (47/90) for patients tested with MCA (Figure 3). In reverse, patients with a CA represented 67/72 (93%) of the confirmed genetic diagnoses. Approximately two-thirds (44/67) of the diagnosed patients had MCA. This group of patients with MCA will be most of the time tested independently of the affected organ systems. Of note, there was no difference in the frequency of affected organ system between diagnosed and undiagnosed patients with an isolated CA nor with the uptake of genetic testing.

Table 3. Time-to-diagnosis for 72 patients with a conclusive genetic diagnosis in relation to the moment genetic testing was started and a genetic diagnosis was confirmed

	Prenatally confirmed diagnosis	Neonatally confirmed diagnosis	Post-neonatally confirmed diagnosis
Suspected genetic disorder prenatally (n=21)	15	1	5
Suspected genetic disorder neonatally (n=35)	N/A	18	17
Suspected genetic disorder post-neonatally (n=16)	N/A	N/A	16

N/A = not applicable

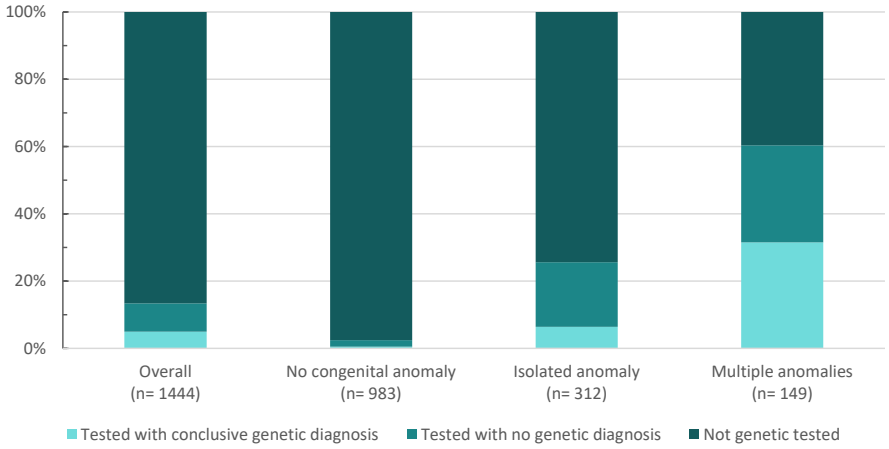

Figure 3. Relative frequencies for the occurrence of congenital anomalies in the total cohort in relation to genetic testing and its outcomes

Types of genetic defects

An overview of types of genetic defects is displayed in Figure 4 and details for all genetic disorders are presented in Supplementary Table 3. Overall, more than half (43/72; 60%)

of the detected genetic defects were single nucleotide variants (SNVs), responsible for monogenic disorders with large genetic and clinical heterogeneity. These genetic disorders were predominantly (35/43; 81%) diagnosed in the post-neonatal period, by unbiased genome wide technologies such as exome sequencing and/or gene panel-based strategies. 15 out of 72 (21%) neonates had an aneuploidy, which were detected prenatally or neonatally by use of technologies such as karyotyping and QF-PCR. Of the latter, the commonly identified genetic disorders were Down´s syndrome/trisomy 21 (10/72; 14%), Patau's syndrome/trisomy 13 (1/72; 1%), Edward's syndrome/trisomy 18 (2/72; 3%), and Turner syndrome (2/72; 3%). The remaining 19% (14/72) of genetic defects (copy number variants (CNVs), uniparental disomy (UPD), and methylation defect) were predominantly detected by genomic microarray and methylation assay.

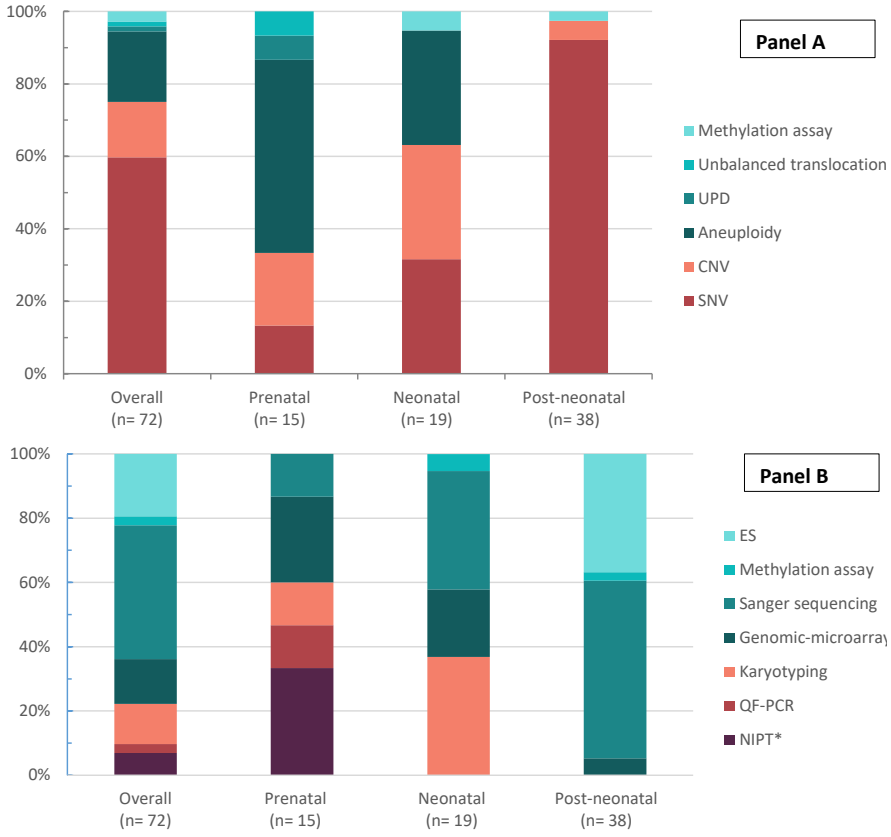

Figure 4. Relative contribution of genetic assay establishing the conclusive genetic diagnosis in relation to moment of testing (panel A) and type of genetic alterations identified (panel B).
*Six abnormal NIPT were confirmed with QF-PCR. UPD = uniparental disomy; CNV = copy number variant; SNV = single nucleotide variant; NIPT = Noninvasive Prenatal Testing; QF-PCR = Quantitative Fluorescent Polymerase Chain Reaction; ES = exome sequencing; NIPT = noninvasive prenatal testing.

3.4 DISCUSSION

In this study we retrospectively evaluated a cohort of 1,444 neonates admitted to the NICU for the presence of genetic disorders and the genetic diagnostic process during the first two years of life. We observed that approximately one third of all neonates at the NICU present with CA, which is often an indication for genetic testing. However, the timeline of genetic testing as part of the diagnostic pathway usually exceeds the neonatal time period. Also, the genetic technologies used during the neonatal period differ from those used in the post-neonatal time frame. In the last years advanced techniques, with higher diagnostic yields, like exome or genome sequencing are used to diagnose patients in the post-neonatal period.

Neonates with congenital malformations indicating a possible genetic disorder comprise a substantial proportion of NICU admissions. Congenital malformations are important signs and should always alert the clinician [3, 7]. The presence of a genetic disorder can easily be missed because of the variable clinical presentation of genetic disorders, often leading to a diagnostic odyssey requiring extensive evaluations, both clinically and genetically [1].

Identifying the genetic cause of a patient's condition puts an end to the diagnostic odyssey, obviating the need for further costly testing. Furthermore, confirmation of a genetic diagnosis has also been shown to alter clinical management [8, 10, 15-17]. This may lead to a reduction in mortality and morbidity related to genetic disorders with onset in newborns. Contrarily, it may facilitate shared decision-making regarding transition to palliative care [8, 18-20]. As an example, in one patient with congenital alveolar capillary dysplasia with misalignment of the pulmonary veins and therapy resistant pulmonary hypertension, the TTD extended beyond one month. Prolonged ineffective cardiorespiratory support could be prevented for this patient given the disastrous prognosis due to the underlying genetic disorder. The timing of diagnosis may have major impact on clinical management of critically ill neonates [17].

Interestingly, we noted that patients suspected of a genetic disorder in the neonatal period were more likely to get a diagnosis faster compared to a resulting suspicion in the post-neonatal phase. The median TTD of patients tested in the prenatal or neonatal period was significantly shorter compared to patients tested in the post-neonatal period. The reasons for this are the shorter turnaround time of the genetic tests used in these patients compared to those who are tested later in life, but also more obvious clinical presentations, like major CA, in neonates which initiated genetic testing compared to for instance developmental disorders or isolated intellectual disability, which only become recognizable later in life [21].

In this study, we identified all patients with CA and determined their genetic diagnostic path throughout their first two years of life. It was observed that 26% (80/312) of infants with an isolated CA were genetically tested, leading to a diagnosis in 25% (20/80) of these patients. Similarly, for patients with MCA, 60% (90/149) of patients received genetic testing, with a diagnostic yield of 52% (47/90). Comparison of the different clinical presentations to determine whether we could identify any clinical indications why some patients with CA were tested, and others were not, did not reveal any specific observations (data not shown). This was not dependent on which organ system was affected. Potential reasons for the reduced uptake of genetic testing in patients with isolated CA or MCA could be unawareness of physicians to order genetic testing, or perceptions of 'too long turnaround times to impact clinical decision-making' and/or parents rejecting genetic evaluation.

Following the above rationale, one may wonder how many patients with CA would have benefited from early genetic testing, thereby reducing their diagnostic odyssey and allowing enhanced patient-tailored medicine. Extrapolation of the data from our cohort and based on the assumption that the genetic diagnostic yield achieved is representative for the remainder of the cohort, an extra 58 (diagnostic yield of 25% in 232 not-tested patients) patients could potentially be diagnosed in the group with an isolated CA, and another 25 (diagnostic yield of 43% in 59 not-tested patients) patients in the sub-cohort with MCA. The diagnostic yield for the group with MCA is corrected for patients with aneuploidies who rarely will not be tested and diagnosed because of the obvious clinical features.

Of note, also in the group of patients without CA, genetic diagnoses were made; retrospective analysis of these patients showed that there were specific clinical indications (mostly later in life) for genetic testing, such as neurodevelopmental delay. As we have limited the follow-up period of our cohort to 2 years of age, it is currently not possible to extrapolate the potential for additional diagnosis in the group of patients without CA. Overall, it is speculated that at least 83 (58 plus 25=6%) patients in our total cohort of 1,444, and 29% (83/291) of not-tested patients with a CA could have likely remained undiagnosed due to a lack in genetic testing.

The limited uptake of genetic testing in daily clinical practice on the NICU patients offers opportunities for improvement, for instance by offering genetic testing to all patients with one or multiple CA. Traditionally, genetic testing has been too time-consuming or perceived to have limited impact on management of the critically ill neonate. Technological advances in recent years have led to the ability to sequence and interpret the entire genome of a neonate in only 1 or 2 days [22, 23]. Whereas many others have already shown that exome or genome sequencing can effectively be used to diagnose

patients in turnaround times required in an acute setting, other clinical utility questions remain unsolved [1, 9, 17-20, 23-26]. This does not only include matters related to genetic consultation, and patient selection, but also socio-economic analyses on cost-effectiveness, and scenario models to determine the most effective strategy to test most, if not all, patients at the NICU.

Ideally, one would analyze these aspects in a prospective parallel study that would offer great insight into the opportunities and potential pitfall of a so-called 'ES or genome sequencing (GS)-first strategy'. Outcome measures should not only focus on quantification of the diagnostic yields via rapid ES, but also on relevant clinical management changes, which are anticipated to range from the initiation of specific patient-tailored supportive management, the transition to palliative care for confirmed lethal conditions to simply refraining from further invasive diagnostic procedures as a consequence of having a final molecular diagnosis [8]. The parental perceptions of WES are also very important and key factors in the process of empowerment must be explored.

Whereas our study has limitations because of its retrospective nature relying only on information available in the patients EMR with only a few years of clinical follow-up, its power is reflected by the systematically assessment of all patients admitted to a level IV NICU for their clinical presentation, genetic testing, and genetic diagnosis obtained. We have motivated the speculation that currently admitted neonates to the NICU are underdiagnosed for disorders of genetic origin. Our results contribute to gaining insight in patient populations that would benefit from ES- or GS-based genetic testing, that not only allows for impact on clinical decision-making in the acute setting but would also limit the diagnostic odyssey of these patients. However, further research is needed to determine the best strategy on whom to offer advanced genetic testing, maximizing the potential of ES or GS in the NICU setting improving the care provided to infants and their families.

REFERENCES

1. Swaggart, K. A., Swarr, D. T., Tolusso, L., He, H., Dawson, D. B., & Suhrie, K. (2019). Making a genetic diagnosis in a Level IV neonatal Intensive care unit population: who, when, how, and at what cost? The Journal of Pediatrics, 213, 211-217.e4. https://doi.org/10.1016/j.jpeds.2019.05.054.
2. Synnes, A., Berry, M., Jones, H. P., Pendray, M., Stewart, S., & Lee, S. K. (2004). Infants with Congenital Anomalies Admitted to Neonatal Intensive Care Units. American Journal of Perinatology, 21(4), 199–207. https://doi.org/10.1055/s-2004-828604.
3. Hudome, S., Kirby, R. S., Senner, J. W., & Cunniff, C. (1994). Contribution of genetic disorders to neonatal mortality in a regional intensive care setting. American Journal of Perinatology, 11(02), 100–103. https://doi.org/10.1055/s-2007-994565.
4. Stevenson, D. A., & Carey, J. C. (2003). Contribution of malformations and genetic disorders to mortality in a children's hospital. American Journal of Medical Genetics - Part A, 126A(4), 393–397. https://doi.org/10.1002/ajmg.a.20409.
5. Weiner, J., Sharma, J., Lantos, J. D., & Kilbride, H. W. (2011). How infants die in the Neonatal Intensive Care Unit. Archives of Pediatrics & Adolescent Medicine, 165(7), 630. https://doi.org/10.1001/archpediatrics.2011.102.
6. Jacob, J., Kamitsuka, M., Clark, R. H., Kelleher, A. S., & Spitzer, A. R. (2015). Etiologies of NICU deaths. Pediatrics, 135(1), e59–e65. https://doi.org/10.1542/peds.2014-2967.
7. Yoon, P. W. (1997). Contribution of birth defects and genetic diseases to pediatric hospitalizations. Archives of Pediatrics & Adolescent Medicine, 151(11), 1096. https://doi.org/10.1001/archpedi.1997.02170480026004.
8. Petrikin, J. E., Willig, L. K., Smith, L. D., & Kingsmore, S. F. (2015). Rapid whole genome sequencing and precision neonatology. Seminars in Perinatology, 39(8), 623–631. https://doi.org/10.1053/j.semperi.2015.09.009.
9. Meng, L., Pammi, M., Saronwala, A., Magoulas, P. L., Ghazi, A., Vetrini, F., Zhang, J., He, W., Dharmadhikari, A. V., Qu, C., Ward, P. A., Braxton, A., Narayanan, S., Ge, X., Tokita, M., Santiago-Sim, T., Dai, H., Chiang, T., Smith, H. S., . . . Lalani, S. R. (2017). Use of exome sequencing for infants in intensive care units. JAMA Pediatrics, 171(12), e173438. https://doi.org/10.1001/jamapediatrics.2017.3438.
10. Wojcik, M. H., Schwartz, T. S., Yamin, I., Edward, H. L., Genetti, C. A., Towne, M. C., & Agrawal, P. B. (2018). Genetic disorders and mortality in infancy and early childhood: delayed diagnoses and missed opportunities. Genetics in Medicine, 20(11), 1396–1404. https://doi.org/10.1038/gim.2018.17.
11. RIVM. Newborn screening (newborn blood spot screening) 2018. Available from: https://www.rivm.nl/en/heel-prick/clinical-picture.
12. Köhler, S., Vasilevsky, N., Engelstad, M., Foster, E. D., McMurry, J. A., Ayme, S., Baynam, G., Bello, S. M., Boerkoel, C. F., Boycott, K. M., Brudno, M., Buske, O. J., Chinnery, P. F., Cipriani, V., Connell, L. E., Dawkins, H., DeMare, L. E., Devereau, A., De Vries, B. B., . . . Robinson, P. N. (2016). The Human Phenotype Ontology in 2017. Nucleic Acids Research, 45(D1), D865–D876. https://doi.org/10.1093/nar/gkw1039.
13. Deden, C., Neveling, K., Zafeiropopoulou, D., Gilissen, C., Pfundt, R., Rinne, T., De Leeuw, N., Faas, B. H. W., Gardeitchik, T., Sallevelt, S. C. E. H., Paulussen, A. D. C., Stevens, S. J., Sikkel, E., Elting, M. W., Van Maarle, M. C., Diderich, K. E. M., Corsten-Janssen, N., Lichtenbelt, K. D., Lachmeijer, G., . . . Van Zelst-Stams, W. a. G. (2020). Rapid whole exome sequencing in pregnancies to identify the underlying genetic cause in fetuses with congenital anomalies detected by ultrasound imaging. Prenatal Diagnosis, 40(8), 972–983. https://doi.org/10.1002/pd.5717.
14. Tavtigian, S. V., Greenblatt, M. S., Harrison, S. M., Nussbaum, R. L., Prabhu, S., Boucher, K. M., & Biesecker, L. G. (2018). Modeling the ACMG/AMP variant classification guidelines as a Bayesian classification framework. Genetics in Medicine, 20(9), 1054–1060. https://doi.org/10.1038/gim.2017.210.
15. Stark, Z., Tan, T. Y., Chong, B., Brett, G. R., Yap, P., Walsh, M., Yeung, A., Peters, H., Mordaunt, D., Cowie, S., Amor, D. J., Savarirayan, R., McGillivray, G., Downie, L., Ekert, P. G., Theda, C., James, P., Yaplito-Lee, J., Ryan, M. M., . . . White, S. (2016). A prospective evaluation of whole-exome sequencing as a first-tier molecular test in infants with suspected monogenic disorders. Genetics in Medicine, 18(11), 1090–1096. https://doi.org/10.1038/gim.2016.1.
16. Meng, L., Pammi, M., Saronwala, A., Magoulas, P. L., Ghazi, A., Vetrini, F., Zhang, J., He, W., Dharmadhikari, A. V., Qu, C., Ward, P. A., Braxton, A., Narayanan, S., Ge, X., Tokita, M., Santiago-Sim, T., Dai, H., Chiang,

T., Smith, H. S., . . . Lalani, S. R. (2017). Use of exome sequencing for infants in intensive care units. JAMA Pediatrics, 171(12), e173438. https://doi.org/10.1001/jamapediatrics.2017.3438.
17. Gubbels, C. S., VanNoy, G. E., Madden, J. A., Copenheaver, D., Yang, S., Wojcik, M. H., Gold, N. B., Genetti, C. A., Stoler, J. M., Parad, R. B., Rumyantsev, S. A., Bodamer, O. A., Beggs, A. H., Juusola, J., Agrawal, P. B., & Yu, T. W. (2020). Prospective, phenotype-driven selection of critically ill neonates for rapid exome sequencing is associated with high diagnostic yield. Genetics in Medicine, 22(4), 736–744. https://doi.org/10.1038/s41436-019-0708-6.
18. Daoud, H., Luco, S. M., Li, R., Bareke, E., Beaulieu, C. L., Jarinova, O., Carson, N., Nikkel, S. M., Graham, G. E., Richer, J., Armour, C. M., Bulman, D. E., Chakraborty, P., Geraghty, M. T., Lines, M. A., Lacaze-Masmonteil, T., Majewski, J., Boycott, K. M., & Dyment, D. A. (2016). Next-generation sequencing for diagnosis of rare diseases in the neonatal intensive care unit. Canadian Medical Association Journal, 188(11), E254–E260. https://doi.org/10.1503/cmaj.150823.
19. Willig, L. K., Petrikin, J. E., Smith, L. D., Saunders, C. J., Thiffault, I., Miller, N., Soden, S. E., Cakici, J. A., Herd, S., Twist, G. P., Noll, A., Creed, M., Alba, P., Carpenter, S. L., Clements, M. A., Fischer, R. T., Hays, J. A., Kilbride, H. W., McDonough, R., . . . Kingsmore, S. F. (2015). Whole-genome sequencing for identification of Mendelian disorders in critically ill infants: a retrospective analysis of diagnostic and clinical findings. The Lancet Respiratory Medicine, 3(5), 377–387. https://doi.org/10.1016/s2213-2600(15)00139-3.
20. Malam, F., Hartley, T., Gillespie, M., Armour, C. M., Bariciak, E., Graham, G. E., Nikkel, S. M., Richer, J., Sawyer, S. L., Boycott, K. M., & Dyment, D. A. (2017). Benchmarking outcomes in the Neonatal Intensive Care Unit: Cytogenetic and molecular diagnostic rates in a retrospective cohort. American Journal of Medical Genetics- Part A, 173(7), 1839–1847. https://doi.org/10.1002/ajmg.a.38250.
21. Walley, N. M., Peña, L., Hooper, S. R., Cope, H., Jiang, Y., McConkie-Rosell, A., Sanders, C., Schoch, K., Spillmann, R. C., Strong, K. A., McCray, A. T., Mazur, P. M., Esteves, C., LeBlanc, K., Wise, A. L., & Shashi, V. (2018). Characteristics of undiagnosed diseases network applicants: implications for referring providers. BMC Health Services Research, 18(1). https://doi.org/10.1186/s12913-018-3458-2.
22. Miller, N., Farrow, E., Gibson, M., Willig, L. K., Twist, G. P., Yoo, B., Marrs, T. W., Corder, S., Krivohlavek, L. A., Walter, A., Petrikin, J. E., Saunders, C. J., Thiffault, I., Soden, S. E., Smith, L. D., Dinwiddie, D. L., Herd, S., Cakici, J. A., Catreux, S., . . . Kingsmore, S. F. (2015). A 26-hour system of highly sensitive whole genome sequencing for emergency management of genetic diseases. Genome Medicine, 7(1). https://doi.org/10.1186/s13073-015-0221-8.
23. Saunders, C. J., Miller, N., Soden, S. E., Dinwiddie, D. L., Noll, A., Alnadi, N. A., Andraws, N., Patterson, M., Krivohlavek, L. A., Fellis, J., Humphray, S., Saffrey, P., Kingsbury, Z., Weir, J. C., Betley, J. R., Grocock, R., Margulies, E. H., Farrow, E., Artman, M., . . . Kingsmore, S. F. (2012). Rapid Whole-Genome sequencing for genetic disease diagnosis in neonatal intensive care units. Science Translational Medicine, 4(154). https://doi.org/10.1126/scitranslmed.3004041.
24. Petrikin, J. E., Willig, L. K., Smith, L. D., & Kingsmore, S. F. (2015). Rapid whole genome sequencing and precision neonatology. Seminars in Perinatology, 39(8), 623–631. https://doi.org/10.1053/j.semperi.2015.09.009.
25. Smith, L. D., Willig, L. K., & Kingsmore, S. F. (2015). Whole-Exome sequencing and Whole-Genome sequencing in critically ill neonates suspected to have Single-Gene disorders. Cold Spring Harbor Perspectives in Medicine, 6(2), a023168. https://doi.org/10.1101/cshperspect.a023168.
26. Petrikin, J. E., Cakici, J. A., Clark, M. M., Willig, L. K., Sweeney, N. M., Farrow, E., Saunders, C. J., Thiffault, I., Miller, N., Zellmer, L., Herd, S., Holmes, A., Batalov, S., Veeraraghavan, N., Smith, L. D., Dimmock, D., Leeder, J. S., & Kingsmore, S. F. (2018). The NSIGHT1-randomized controlled trial: rapid whole-genome sequencing for accelerated etiologic diagnosis in critically ill infants. Npj Genomic Medicine, 3(1). https://doi.org/10.1038/s41525-018-0045-8.

SUPPLEMENTARY TABLES

Supplementary Table 1. Clinical characteristics of the included patients
Supplementary Table 1 can be found online at
https://doi.org/10.1007/s00431-021-04213-w

Supplementary Table 2. Overview of types of genetic tests performed, including time period
Supplementary Table 2a. Types of genetic tests
Supplementary Table 2b. Time period of genetic diagnostic trajectory

Supplementary Table 2 can be found online at
https://doi.org/10.1007/s00431-021-04213-w

Supplementary Table 3. Details of genetic disorders
Supplementary Table 3a. Clinical details of 72 patients who received a conclusive genetic diagnosis
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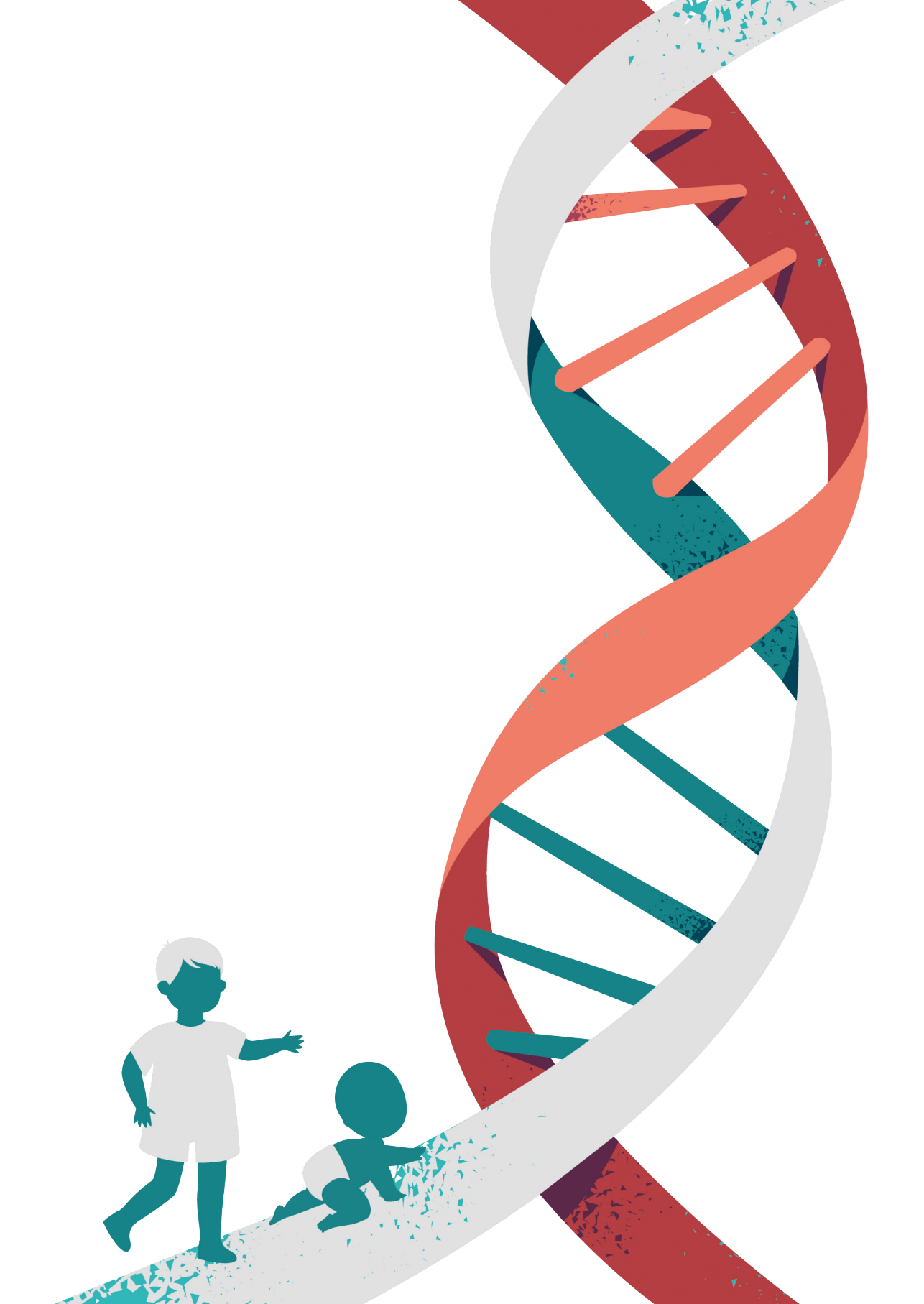
Supplementary Table 3 can be found online at
https://doi.org/10.1007/s00431-021-04213-w



CHAPTER 4

Medical costs of children admitted to the neonatal intensive care unit: the role and possible economic impact of exome sequencing in early diagnosis

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ABSTRACT

It has been estimated that at least 6.0% of neonates admitted to the Neonatal Intensive Care Unit remains genetically undiagnosed because genetic testing is not routinely performed. The objective of this study is to provide an overview of average healthcare costs for patients admitted to the Neonatal Intensive Care Unit and to assess possible impact of implementing exome sequencing (ES) on these total healthcare costs. Hereto, we retrospectively collected post-natal healthcare data of all patients admitted to the level IV neonatal intensive care unit at the Radboudumc (October 2013–October 2015) and linked unit costs to these healthcare consumptions. Average healthcare costs were calculated and a distinction between patients was made based on performance of genetic tests and the presence of congenital anomalies. Overall, on average €26,627 was spent per patient. Genetic costs accounted for 2.3% of all costs. Healthcare costs were higher for patients with congenital anomalies compared to patients without congenital anomalies. Patients with genetic diagnostics were also more expensive than patients without genetic diagnostics. We next modelled four scenarios based on clinical preselection. First, when performing trio-ES for all patients instead of current diagnostics, overall healthcare costs will increase with 22.2%. Second, performing trio-ES only for patients with multiple congenital anomalies will not result in any cost changes, but this would leave patients with an isolated congenital anomaly untested. We therefore next modelled a scenario performing trio-ES for all patients with congenital anomalies, increasing the average per patient healthcare costs by 5.3%. This will rise to a maximum of 5.5% when also modelling for an extra genetic test for clinically selected patients to establish genetic diagnoses that are undetectable by ES. In conclusion, genetic diagnostic testing accounted for a small fraction of total costs. Implementation of trio-ES as first-tier test for all patients with congenital anomalies will lead to a limited increase in overall healthcare budget, but will facilitate personalized treatments options guided by the diagnoses made.

4.1 INTRODUCTION

Genetic disorders are of great impact in the wellbeing of an individual. For instance, in Europe, 23.9 per 1,000 births between 2003 and 2007 have a congenital anomaly caused by a genetic disorder [1]. The prevalence of genetic disorders within the neonatal intensive care unit (NICU) is not exactly known and can be missed easily in a neonatal setting [2]. Earlier research has shown that a large part of patients admitted to the NICU consists of patients with a genetic disorder and 30-50% of these genetic disorders results in neonatal and infant deaths [3-9]. It has been shown that 13.7% of the patients admitted to the NICU suffer from an isolated congenital anomaly (CA) or multiple congenital anomalies (MCAs) [8, 10]. As CAs often have a genetic origin, genetic diagnostic testing in these patients may help to obtain a diagnosis. We previously confirmed a correlation between a conclusive genetic diagnosis and the presence of CAs [11]. We additionally showed that not all neonates with CA receive genetic testing, leaving at least 6.0% of neonates without a diagnosis [11]. This finding indicates the need for improvement of genetic diagnostic research in order to diagnose these patients, decrease the time-to-diagnosis with the possibility of starting treatment earlier [11-12]. The introduction of genetic tests for all patients with CAs albeit clinically relevant, will have economic effects that need to be clarified.

The advent of novel genetic technologies, including exome sequencing (ES) and genome sequencing (GS), have made it possible to investigate the exome or genome, without the need for a priori knowledge about a suspected underlying cause of the disease [13]. Implementing ES or GS at an early stage during the diagnostic trajectory can result in a timely diagnosis. Once the diagnosis has been established, the appropriate type of care can be initiated. Timely genetic testing compared to “delayed” diagnostics, shows a decrease in healthcare consumption [13].

These novel genetic technologies are very promising for their clinical added value. However, implementation of novel genetic technologies also may come at high costs. Therefore, the latter impact should deliberately be discussed and adequately analyzed. Previous research stated that implementation of ES and GS might be cost-effective in the future for critically ill newborn infants [3, 12, 14-15]. The evidence is very scarce and it is questionable whether outcomes and cost savings can be extrapolated between different patient groups and different countries.

To decide on implementing new diagnostic approaches like ES or GS, it is essential to put additional costs into perspective. The main objective of this study is to retrospectively calculate the uptake of genetic testing and associated costs for patients admitted to the NICU. The secondary objective is to assess the possible impact of implementing ES on the total healthcare costs.

4.2 MATERIALS AND METHODS

Study participants and healthcare data collection

In order to include a long follow-up period and to start before the introduction of WES as part of the diagnostic trajectory, patients were included in this retrospective study if they were admitted to the level IV NICU at the Radboud University Medical Center (Radboudumc), Nijmegen, the Netherlands, between October 2013 and October 2015. The clinical details of this cohort are presented in Marouane et al. [11]. In brief, the study population consisted of 312 neonates presented with an isolated CA and 149 with MCA. When represented by 21 organ systems in human phenotype ontology, anomalies were found in all organ systems, with cardiovascular system (159 neonates), genitourinary system (76 neonates), and growth abnormalities (70 neonates) being affected mostly. For neonates with MCA, on average 2.8 organ systems were affected. In this cohort, no correlation was found between the severity of the CA (isolated or multiple) and the uptake of genetic testing [11]. Patients were excluded from this study in case (i) the patient was admitted to the NICU due to results from the neonatal blood spot screening program; (ii) genetic diagnostic testing was performed to investigate the presence of a known familial mutation; or (iii) no healthcare data could be retrieved. We retrospectively collected all available healthcare data from hospital information systems and patient records. We collected all healthcare data until April 4th, 2019. On this date, data was collected. This study was approved by the Medical Research Ethics Committee Arnhem/Nijmegen under file number 2016-2486/NL57511.091.16.

Data analysis

In this study, the total number (and percentage) of females and males and the average age in days at the moment of data collection (including standard deviation) were calculated. Follow-up time was calculated as the number of days between the first hospital visit until the moment of data-collection (April 4th, 2019). In this analysis, we made a distinction between patients who did and those who did not receive genetic testing, and those with and without CAs (Figure 1). Details on the genetic tests performed per patient are described in detail by Marouane et al. [11] and presented in summary in Supplementary Table A.

The outcome measures of this study were healthcare resource use and costs related to this healthcare resource use. Healthcare data was divided into seven types of costing categories: (i) hospitalization; (ii) consultations; (iii) diagnostics; (iv) medication; (v) genetics; (vi) surgery; and (vii) other, which consists of all other healthcare activities which cannot be categorized into one of the previous categories. Genetic costs consisted of costs related to both genetic counseling and genetic diagnostic testing. Unit prices were retrieved from the Dutch Healthcare Authority (Nederlandse Zorgautoriteit; NZA), the cost-manual of the National Healthcare Institute (Zorginstituut Nederland; ZIN) and

literature research and linked to the corresponding healthcare activities [16-20]. All unit prices were converted to the same index year (2020). Descriptive and scenario analyses were performed in R (version 4.0.3) [17].

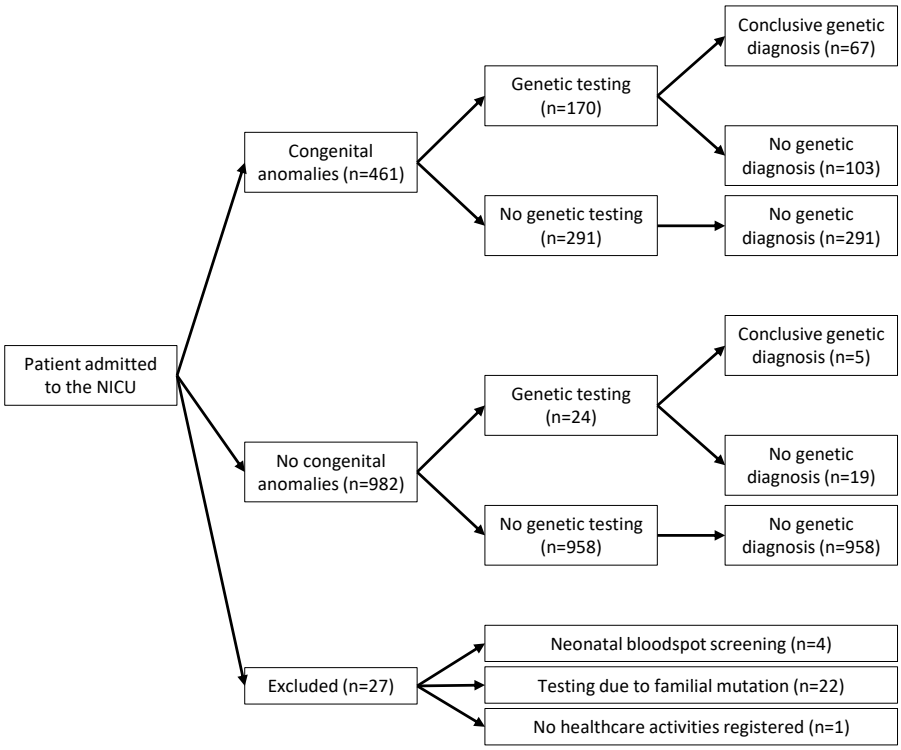


Figure 1. Patients included in this study.
NICU = neonatal intensive care unit.

Descriptive analysis of current healthcare costs

1) **Average healthcare costs.** This analysis was performed with the available healthcare data, for all patients divided in the different costing categories. For all categories, the mean, median and range (minimum and maximum) of costs, total number of healthcare activities and the average unit price were calculated.
2) **Genetic diagnostic testing vs. no genetic diagnostic testing.** The average healthcare costs for patients who received genetic diagnostic testing were compared to patients who did not receive genetic diagnostic testing. For each costing category, the average costs per patient were calculated, including mean, median and range (minimum and maximum). Furthermore, for each costing

category, the number of units and the average unit price was calculated. The minimum follow-up period of patients included in this study was 730 days. Therefore, in order to ensure comparability between groups, follow-up time included in the analysis for these patients was 730 days starting on the first day of admission to the NICU.

3) **Isolated CAs vs. MCAs vs. no CAs.** We also compared the average healthcare costs for patients with an isolated CA, MCAs and patients without CAs. We calculated average costs per patient, including mean, median and range (minimum and maximum), and the number of healthcare activities including their unit costs. For this sub-analysis, follow-up time was also limited to 730 days starting on the day of admission to the NICU to ensure comparability between groups.

Scenario analysis

The presence of CAs is one of the most obvious reasons to suspect a genetic defect that underlies a disorder. In order to ensure comparability between groups, patients with a prenatal diagnosis were excluded from the scenario analyses. The following four scenario analyses were performed:

1) **Implementing ES as first-tier test for all patients admitted to the NICU.** The average healthcare costs and the total costs related to genetic testing in case all patients admitted to the NICU would receive ES were estimated and compared to the current costs (Figure 2, scenario A). For patients who did receive genetic diagnostic tests, all costs related to these genetic diagnostic costs were excluded from the results since it was assumed that performing ES would result in the same diagnosis and thus replaces the whole standard diagnostic trajectory. Healthcare activities which were included in the ES scenario are pre-test genetic counseling, performing (trio-)ES and post-test genetic counseling. The total costs related to this ES scenario were calculated by multiplying the total number of patients admitted to the NICU by €2,892, i.e. the costs of pre- and post-test genetic counseling (€1,089 [16-20]) and per-sample costs of ES (€1,803 (16)). Costs related to performing trio-ES were €6,498 (pre- and post-test genetic counseling and 3 times the per-sample costs of ES). The current costs of genetics were compared to the costs resulting from this scenario analysis.

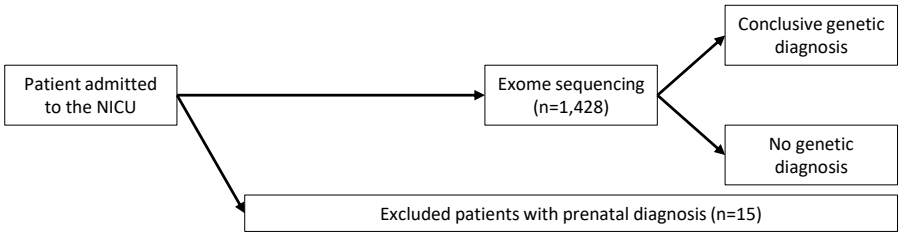


Figure 2. Scenario analyses. Scenario A.
NICU = neonatal intensive care unit.

2) **Implementing ES as first-tier test for all patients with CAs admitted to the NICU.** The average healthcare costs and the total costs related to genetic testing in case all patients with CAs and admitted to the NICU receive ES were estimated and compared to the current costs (Figure 2, scenario B). As with scenario analysis A, current costs for genetic testing were excluded from the scenario in which all patients with CAs receive (trio-)ES and total genetic costs were calculated by using the same formula. In the end, costs related to performing ES for all patients with CAs were compared to the genetic costs related to the standard and current genetic diagnostic trajectory.

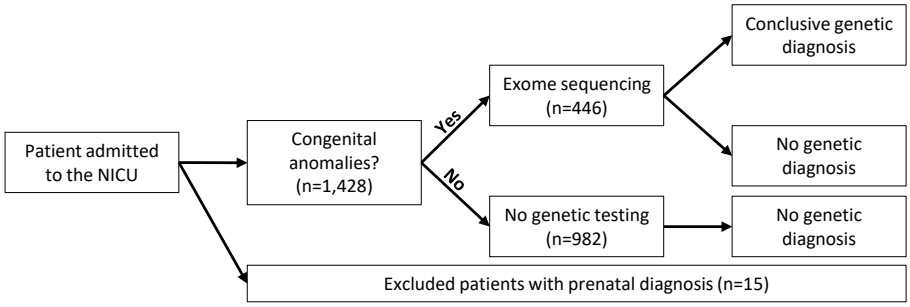


Figure 2. Scenario analyses. Scenario B.
NICU = neonatal intensive care unit.

3) **Implementing ES as first-tier test for all patients with MCAs.** In scenario C, current healthcare costs were compared to the costs in case all patients with MCAs received ES. The average total healthcare costs and genetic costs were calculated just like scenario A and B.

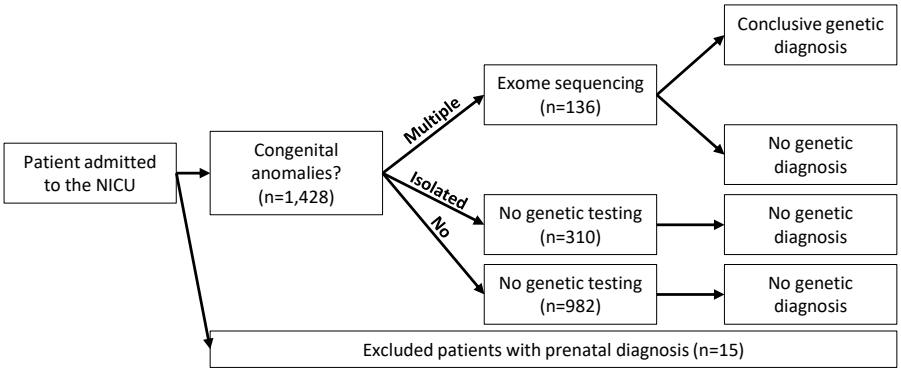


Figure 2. Scenario analyses. Scenario C.
NICU = neonatal intensive care unit.

4) **Implementing ES as first-tier test for patients with CAs, including additional genetic tests.** Because of technical limitations, ES is not able to detect all clinically relevant variation, such as for instance methylation defects. A fourth scenario was performed in which a correction factor was added to the costs associated with additional diagnostic testing (Figure 2, scenario D). Hereto, the genetic tests performed, and diagnosis obtained in this cohort were assessed from Marouane et al. [11]. Per assay, it was determined whether the type of variants they assess, can also (technically) be detected from ES data. For patients with a conclusive genetic diagnosis, it was assessed whether it is possible to find the same diagnosis by performing ES or whether an additional genetic test is required. Average per patient costs of these additional tests were calculated and added to the ES scenario in which ES was implemented as first-tier test for all patients with CAs.

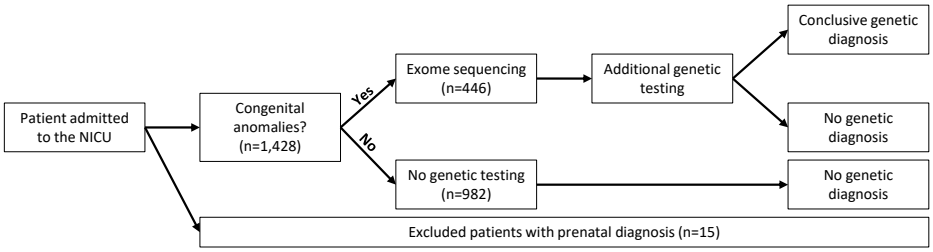


Figure 2. Scenario analyses. Scenario D.
NICU = neonatal intensive care unit.

4.3 RESULTS

Study participants

Overall, 1470 patients were admitted to the NICU of the Radboudumc between October 2013 and October 2015. In total, 27 patients were excluded from the data analysis, resulting in a study population of 1,443 patients (Figure 1). In the scenario analysis, another 15 patients were excluded due to a prenatal genetic diagnosis. There were 610 females (42.3%) and 833 boys (57.7%), with a mean age of 1,650 (±209) days at the moment of data collection. Of these 1,443 neonates, the average follow-up time was 1,614 (±209) days. An overview of follow-up time per patient is shown in Supplementary Table B.

Descriptive analysis of current healthcare costs

1) **Average healthcare costs.** For all 1,443 included patients, the average costs spend on healthcare were €26,627 per patient (Table 1). Hospitalization accounted for the largest part (84.1%) of all healthcare costs, which was on average €22,382 per patient. An overview of all cost categories and its corresponding costs can be seen in Table 1.

   Of 1,443 patients included in this study, 194 patients (13.4%) received genetic diagnostic testing. In total, 410 genetic diagnostic tests were performed, totaling to an average of €616 per patient, and accounting for 2.3% of the total costs for all 1,443 patients together.

Table 1. Total healthcare costs per patient (n = 1,443)

	Mean (€)	Percentage of total (%)	Median (€)	Min (€)	Max (€)	Units (n)	Unit costs (€)
Hospitalization	22,382	84.1	7,178	0	587,353	27,084	1,192
Consultations	85	0.3	0	0	3,051	3,356	36
Diagnostics	1,476	5.5	515	0	22,600	220,165	10
Medication	127	0.5	0	0	160,961	1,464	125
Genetics[a]	616	2.3	0	0	14,064	1,269	700
Other	1,911	7.2	457	0	35,039	36,224	76
Surgery	31	0.1	0	0	15,780	896	49
Total	26,627		9,665	69	630,689	290,458	132

[a] Includes all costs related to genetics (i.e. genetic counseling, genetic diagnostic tests)

2) **Genetic diagnostic testing vs. no genetic diagnostic testing.** Table 2 shows the costs of healthcare use for the different categories in patients who received genetic diagnostic tests and patients who did not receive genetic diagnostic tests.

   For patients who did receive genetic diagnostic testing, the average costs spend on healthcare was €43,804 per patient. 5.8% (€2,523 per patient) of these costs consisted of costs related to genetics.

   For patients who did not receive genetic diagnostic testing, the total costs spend on healthcare were €18,404 per patient. Of these costs, 0.5% (€99 per patient) were related to genetics, i.e. genetic consultation and evaluation without performing any genetic diagnostic tests.

   For both patient groups, the main part of the total healthcare costs consisted of costs related to hospitalization, 80.0% (€35,055 per patient) and 87.0% (€16,007 per patient) of all costs for patients with and without genetic diagnostic testing respectively.

Table 2. Average healthcare costs per child per category for patients who did or did not receive genetic diagnostic tests (n = 1,443)[a]

	Mean (€)	Percentage of total (%)	Median (€)	Min (€)	Max (€)	Units (n)	Unit costs (€)
Patients without genetic diagnostics (n = 1,249)							
Hospitalization	16,007	87.0	5,556	0	501,834	16,760	1,193
Consultations	35	0.2	0	0	1,760	1,156	38
Diagnostics	984	5.3	334	0	20,325	133,654	9
Medication	144	0.8	0	0	160,961	1,117	160
Genetics[b]	99	0.5	0	0	6,449	323	384
Other	1,123	6.1	211	0	31,100	15,376	91
Surgery	11	0.1	0	0	1,565	452	32
Total	18,404		6,606	0	527,393	168,838	136
Patients who received genetic diagnostics (n = 194)							
Hospitalization	35,055	80.0	16,125	0	471,572	5,499	1,237
Consultations	249	0.6	117	0	2,073	1,264	38
Diagnostics	2,505	5.7	1,501	0	16,024	45,304	11
Medication	2	0.0	0	0	302	233	2
Genetics[b]	2,523	5.8	1,745	0	12,692	589	831
Other	3,342	7.6	2,409	0	25,534	9,697	67
Surgery	127	0.3	0	0	15,643	254	97
Total	43,804		24,230	569	501,537	62,840	135

[a] From date of admission to the neonatal intensive care unit until 730 days after this admission date; [b] Includes all costs related to genetics (i.e. genetic counseling, genetic diagnostic tests)

3) **Isolated CAs vs. MCAs vs. no CAs.** The average healthcare costs for patients with and without CAs are shown in Table 3. Total healthcare costs were €27,350 and €53,686 per patient for patients with isolated CAs and MCAs respectively. For patients without CAs, these total healthcare costs were on average €15,210 per patient.

   For patients with isolated CAs, costs related to hospitalization accounted for 79.7% (€21,808 per patient) and costs related to genetics accounted for 2.4% (€665 per patient) of all costs. For patients with MCAs, 83.4% (€44,781 per patient) of the costs was related to hospitalization and 3.8% (€2,036 per patient) to genetics. 89.1% (€13,548 per patient) of the costs and 0.7% (€104) of the costs were related to hospitalization and genetics respectively for patients without CAs.

Table 3. Average healthcare costs per child per category for patients with isolated, multiple or without congenital anomalies (n = 1,443)[a]

	Mean (€)	Percentage of total (%)	Median (€)	Min (€)	Max (€)	Units (n)	Unit costs (€)
Patients without congenital anomalies (n = 982)							
Hospitalization	13,548	89.1	4,400	0	242,384	10,987	1,212
Consultations	19	0.1	0	0	900	484	38
Diagnostics	781	5.1	277	0	14,230	88,235	9
Medication	2	0.0	0	0	1,095	325	7
Genetics[b]	104	0.7	0	0	9,601	233	439
Other	751	4.9	136	0	24,746	9,488	78
Surgery	4	0.0	0	0	936	176	24
Total	15,210		5,418	0	267,073	109,928	136
Patients with an isolated congenital anomaly (n = 312)							
Hospitalization	21,808	79.7	11,461	0	501,834	6,000	1,134
Consultations	116	0.4	39	0	2,073	946	38
Diagnostics	1,694	6.2	948	0	15,503	49,308	11
Medication	568	2.1	0	0	160,961	685	259
Genetics[b]	665	2.4	0	0	11,888	287	723
Other	2,486	9.1	1,059	0	25,534	6,949	112
Surgery	14	0.1	0	0	914	269	16
Total	27,350		15,481	10	527,393	64,444	132
Patients with multiple congenital anomalies (n = 149)							
Hospitalization	44,781	83.4	18,987	298	471,572	5,272	1,266
Consultations	252	0.5	98	0	1,838	990	38
Diagnostics	2,808	5.2	1,543	0	20,325	41,415	10
Medication	3	0.0	0	0	302	340	1
Genetics[b]	2,036	3.8	872	0	12,692	392	774
Other	3,601	6.7	2,441	0	31,100	8,636	62
Surgery	204	0.4	0	0	15,643	261	116
Total	53,686		25,870	2,200	501,537	57,306	140

[a] From date of admission to the neonatal intensive care unit until 730 days after this admission date; [b] Includes all costs related to genetics (i.e. genetic counseling, genetic diagnostic tests)

Scenario analysis

Since trio-ES approach will be the most relevant as genetic diagnostic trajectory in this study population, only results regarding trio-ES (Table 4) will be discussed in this paper. Results regarding implementing single-ES can be found in Supplementary Table C.

1) **Implementing ES as first-tier test for all patients admitted to the NICU.** In case trio-ES was performed for all 1,428 patients admitted to the NICU (Figure 2, scenario A), total genetic costs increased to €6,498 per patient, which is an increase in costs of 949.8% (Table 4). The average total healthcare costs increased 22.2% (€32,361 instead of €26,482 on average per patient).
2) **Implementing ES as first-tier test for all patients with CAs and admitted to the NICU.** 446 patients (31.2%) were admitted to the NICU with CAs and were included in this scenario analysis (Figure 2, scenario B). Of these patients, 291 patients did not receive any genetic diagnostic testing (65.2%). The total healthcare costs related to genetic diagnostic testing in case trio-ES was performed for all these patients (n = 446) were on average €2,207 per patient (n = 1,428). Compared to the regular diagnostic trajectory this was an increase in costs of 227.4% (on average €1,408 per patient extra, see Table 4). The average total healthcare costs increased with 5.3% (€27,893 per patient).
3) **Implementing ES as first-tier test for patients with MCAs.** In total, 136 patients had MCA. Replacing the traditional diagnostic trajectory by performing trio-ES for patients with MCA did not result in a change in average genetic diagnostic costs and overall healthcare costs (Table 4).
4) **Implementing ES as first-tier test for patients with CAs, including additional genetic tests.** Due to technical limitations, not all variant types can be detected from ES data. We therefore assessed the use of assays detecting variation that escape detection by ES (Table 5), and identified four assays providing additional genetic information: fluorescence in situ hybridization (FISH), Methylation assays, hemoglobinopathies, and testing for fragile sites. Per patient, an overview of the genetic disorders, diagnostic tools, variants and detectability by ES is provided in Supplementary Table D. For the tests of which the results are not detectable by ES, we subsequently determined the average use in the cohort and determined the associated costs. On average, this scenario increased the costs by an additional €105 per patient, resulting in an average total healthcare costs increase of 5.5% (€27,926 per patient).

Table 4. Average costs per patient for scenario A, B, C and D implementing trio-ES

	Current costs[a] (€)	Scenario A (€) Trio-ES[b]	Scenario B (€) Trio-ES[b]	Scenario C (€) Trio-ES[b]	Scenario D (€) Trio-ES[b]
Costs related to genetic testing					
Patients with multiple congenital anomalies (n = 136)	2,816	6,498	6,498	6,498	6,603
Patients with isolated congenital anomaly (n = 310)	954	6,498	6,498	0	6,603
Patients without congenital anomalies (n = 982)	208	6,498	0	0	0
Total (n = 1,428)	**619**	**6,498 (+949.8%)**	**2,027 (+227.4%)**	**619 (+0.0%)**	**2,062 (+333.2%)**
Overall healthcare costs					
Patients with multiple congenital anomalies (n = 136)	69,170	72,852	72,852	72,852	72,957
Patients with isolated congenital anomaly (n = 310)	33,414	38,958	38,958	32,460	39,063
Patients without congenital anomalies (n = 982)	18,382	24,671	18,173	18,173	18,173
Total (n = 1,428)	**26,482**	**32,361 (+22.2%)**	**27,893 (+5.3%)**	**26,482 (+0.0%)**	**27,926 (+5.5%)**

ES = exome Sequencing; [a] Without taking into account the differences in follow-up time and excluding patients with a prenatal diagnosis; [b] Costs include pre-test genetic counseling, trio-ES and post-test genetic counseling

Table 5. Genetic test (result) and detectability from ES

Type of genetic test	Reason of use	Total patients receiving the test (from n=194)	Average number of tests per patient	Variant(s) technically detectable from ES
NIPT	Trisomy 13; trisomy 18; trisomy 21	6	0.03	*Out of scope – prenatal test*
QF-PCR	Trisomy 13; trisomy 18; trisomy 21; sex chromosomal aberrations	45	0.23	Yes
Karyotype	(Confirmation of) chromosomal aberrations	39	0.20	Yes
Genomic microarray	CNVs	112	0.58	Yes
Prenatal gene panel (Noonan syndrome / Cystic Fibrosis)	SNVs, indels and CNVs in selected genes	2	0.01	*Out of scope – prenatal test*
Sanger	SNVs and indel in targeted gene (based on clinical presentation)	57	0.29	Yes
FISH	(confirmation of) CNVs and testing for balanced aberrations	8	0.04	Possibly
ES	SNVs, indels and CNVS in disease gene panel(s)	55	0.28	Yes
Methylation assay	Hypo/hypermethylation of targeted gene(s) (based on clinical presentation)	8	0.04	Possibly
Hemoglobinopathy	Assessment of α- and β-globin gene clusters	2	0.01	No
Chromosomal Breakage Syndrome	Assessment of chromosomal fragile sites	5	0.03	No

NIPT = noninvasive prenatal testing; QF-PCR = quantitative fluorescence polymerase chain reaction; FISH = fluorescence in situ hybridization; ES = Exome Sequencing; CNVs = copy number variations; SNVs = single nucleotide variations.

4.4 DISCUSSION

We presented an overview of the average healthcare costs of patients admitted to the NICU. We estimated the economic impact of the inclusion of ES as part of the diagnostic trajectory on the resources spent on genetic testing and on the overall healthcare costs. We modelled multiple scenarios to find a balance between making maximal benefits of the use of ES in this patient population, while limiting the socio-economic impact on the healthcare system. Our results showed that care for patients who received genetic diagnostic tests (€43,804 per patient) was more expensive compared to care for patients who did not receive genetic diagnostic testing (€18,404 per patient). This has been calculated for the first two years after the patients were admitted to the NICU. Of these costs, €2,523 (5.8% of all costs) and €99 (0.5% of all costs) was spent on genetic testing, respectively. Apart from spending more budget on genetic testing for the patients with CAs, the main difference was attributed to costs for hospitalization and consultations. This was in line with results obtained by others, showing that more complex patients

often have higher healthcare costs [22]. We observed a similar trend between patients with isolated CAs and MCAs: care for patients with MCAs was more expensive than for patients with isolated CAs (€53,686 vs. €27,350 per patient). Care for patients without CAs was the least expensive (€15,210 per patient).

Of the 57 patients that received a conclusive genetic diagnosis and were included in the scenario analyses, 52 presented CAs, highlighting that such anomalies can have a genetic origin [11]. In the total cohort, 461 patients presented with CAs, but not all patients received genetic testing, which by extrapolation of diagnostic yield, left 83 patients (6% of the total cohort) without a genetic diagnosis [11]. An argument of not testing in these patients is often related to the long turnaround times. Since these have been drastically reduced by the introduction of ES, we modelled four scenarios to determine the anticipated impact on diagnostic yield and healthcare costs for wider spread implementation in a NICU setting.

In scenario A, all 1,428 patients would receive genetic diagnostic testing by ES. Compared to the current costs, genetic costs increased with 949.8% and overall healthcare costs with 22.2%. Although costs will increase significantly, testing all patients will allow to identify possible diagnosis identifiable by ES (~95% of all diagnosis) in the cohort in one test, at the start of the diagnostic trajectory. Moreover, testing allows to identify an extrapolated number of diagnoses that currently remain undetected because patients are not subjected to diagnostic testing. Since only 5 diagnoses were made in patients without CAs [11], one might wonder whether clinical preselection on who should receive ES based on having CAs is not a more economically sustainable option.

We therefore modelled scenario C, where ES was only performed for patients with MCAs, as this clinical sub-cohort of NICU patients had relative highest diagnostic yield [11]. Testing all 136 patients with MCAs by trio-ES, but refraining from genetic testing in all other patients (n = 1,428), resulted in no change in healthcare costs. Whereas there may be an economic benefit when introducing trio-ES for patients with MCAs, this scenario seems unethical as the diagnostic yield in patients with isolated CAs in this cohort is 5.8% (18 out of 310 patients). Hence, at cohort level, this scenario would leave up to half of all patients with a rare disease undiagnosed.

Scenario B, allowing to perform ES for those patients with either isolated CAs or MCAs, seemed as such most beneficial. The costs related to genetic testing increased with 227.4% for trio-ES, but the average total healthcare costs increased with 5.3% (€1,411). Although the costs are higher compared to the current diagnostic trajectory, this would allow the detection of ~95% of all genetic diagnoses in the cohort. This scenario includes both the diagnoses established in the current situation, but also the diagnoses of those

patients who currently remain untested despite a clinical presentation suspicious to be of genetic origin. This approach would however miss the genetic diagnosis in the 5 of 982 patients without CAs, and those that were obtained via another assay that cannot be replaced by ES (4 diagnoses). The 5 patients without CA now received a conclusive genetic diagnosis after performing genetic testing well after the neonatal period because of (neuro)developmental delay. It might, however, be expected that these patients would still receive genetic testing later in life, similar to the current situation, because of developmental delay [11], but would not immediately benefit from wide implementation of rapid ES in the NICU setting. For the 4 patients whose diagnoses would be delayed by only offering ES, we performed scenario D.

In scenario A, B and C, it was assumed that ES provided the same results as the standard diagnostic trajectory. To not withhold patients their timely diagnosis, we modelled Scenario D, in which we performed ES for all patients with CAs (isolated and MCA). We also included extra dedicated tests based on clinical presentation, to capture variants undetectable by ES, and thus still being able to detect all diagnoses made in the cohort. This scenario showed an increase in genetic costs of 333.2%, and a total healthcare costs increase of 5.5% (€1,444) compared to the standard diagnostic trajectory. In practice, we expect this scenario to be the most realistic approach to future care. Albeit, it could be argued that those additional tests are performed only in patients with a negative ES result, all tests are likely to be performed in parallel because the turnaround time is crucial.

A possible limitation of this study was that the diagnostic trajectory was not completed for all patients included in the scenario analysis. Although we did not include any restrictions regarding follow-up length, total healthcare costs used in these scenario analyses are probably higher due to an increase in the amount of healthcare costs in case a longer follow-up period is included. This can also have impact on the increase in costs when healthcare costs are compared to the ES-scenarios. The same holds for the calculated costs related to surgery and medication, which might also be underestimated. Unfortunately, it was not possible to collect all unit costs taking into account the amount of medication prescribed and unit costs related to surgery. Therefore, which is also a strength of this study, the results of this study showed the maximum increase in costs when ES is performed. If this study is repeated after a certain amount of time, results of the scenario analyses will probably be more positive (i.e. less increase in costs due to increase in current healthcare costs and decrease in costs related to WES). According to earlier research, it is very valuable that patients were followed over time for several years [23]. The genetic diagnostic trajectory can be very long and costly, so including a longer follow-up period in an economic evaluation provides a more accurate overview of the actual costs.

In this study, costs related to prenatal diagnostic testing were not taken into account. This aspect requires dedicated studies, especially with the increasing uptake of rapid WES in prenatal settings [24-25]. Noteworthy however is that prenatal rapid ES is mostly performed based on ultrasound abnormalities, and thus, likely representing the patients who after birth, are admitted to NICU. This group is therefore expected to create a shift in the moment ES is performed, but will not create an additional group of patients. Regardless of the time of testing, the implementation of ES has the potential to influence the diagnostic trajectory and the treatment and care after a diagnosis is established and consequently the costs. Ideally, a prospective follow-up study is performed, in which patients receive current diagnostics and trio-ES to see what the exact clinical and economic impact is of implementing ES.

In conclusion, genetic diagnostic testing in a NICU patient cohort accounts for a small fraction of total costs. Only half of patients whose clinical presentation is suggestive of a genetic disorder, are currently being tested. We showed that with limited increase in overall healthcare budget on this cohort, all patients presenting with CAs can be tested by trio-ES. This will not only increase the overall diagnostic yield of this cohort, but may also allow for improved personalized treatments options guided by the diagnoses made.

REFERENCES

1. Dolk, H., Loane, M., & Garne, E. (2010). The prevalence of congenital anomalies in Europe. In Advances in Experimental Medicine and Biology (pp. 349–364). https://doi.org/10.1007/978-90-481-9485-8_20.
2. Hudome, S., Kirby, R. S., Senner, J. W., & Cunniff, C. (1994). Contribution of genetic disorders to neonatal mortality in a regional intensive care setting. American Journal of Perinatology, 11(02), 100–103. https://doi.org/10.1055/s-2007-994565.
3. Borghesi, A., Mencarelli, M. A., Memo, L., Ferrero, G. B., Bartuli, A., Genuardi, M., Stronati, M., Villani, A., Renieri, A., & Corsello, G. (2017). Intersociety policy statement on the use of whole-exome sequencing in the critically ill newborn infant. Italian Journal of Pediatrics, 43(1). https://doi.org/10.1186/s13052-017-0418-0.
4. Wojcik, M. H., Schwartz, T. S., Yamin, I., Edward, H. L., Genetti, C. A., Towne, M. C., & Agrawal, P. B. (2018). Genetic disorders and mortality in infancy and early childhood: delayed diagnoses and missed opportunities. Genetics in Medicine, 20(11), 1396–1404. https://doi.org/10.1038/gim.2018.17.
5. Simpson, C., Ye, X. Y., Hellmann, J., & Tomlinson, C. (2010). Trends in Cause-Specific mortality at a Canadian outborn NICU. Pediatrics, 126(6), e1538–e1544. https://doi.org/10.1542/peds.2010-1167.
6. Costa, S., Rodrigues, M., Centeno, M. J., Martins, A., Vilan, A., Brandão, O., & Guimarães, H. (2010). Diagnosis and cause of death in a neonatal intensive care unit - How important is autopsy? Journal of Maternal-fetal & Neonatal Medicine, 24(5), 760-763. https://doi.org/10.3109/14767058.2010.520047.
7. Wen, S. W., Liu, S., Joseph, K. S., Rouleau, J., & Allen, A. C. (2000). Patterns of infant mortality caused by major congenital anomalies. Birth Defects Research, 61(5), 342–346. https://doi.org/10.1002/(sici)1096-9926(200005)61:5.
8. Weiner, J., Sharma, J., Lantos, J. D., & Kilbride, H. W. (2011). How infants die in the Neonatal Intensive Care Unit. Archives of Pediatrics & Adolescent Medicine, 165(7), 630. https://doi.org/10.1001/archpediatrics.2011.102.
9. Tyson, J. E., & Kennedy, K. A. (2002). Variations in mortality rates among Canadian neonatal intensive care units: interpretation and implications. PubMed, 166(2), 191–192. https://pubmed.ncbi.nlm.nih.gov/11826942.
10. Synnes, A., Berry, M., Jones, H. P., Pendray, M., Stewart, S., & Lee, S. K. (2004). Infants with Congenital Anomalies Admitted to Neonatal Intensive Care Units. American Journal of Perinatology, 21(4), 199–207. https://doi.org/10.1055/s-2004-828604.
11. Marouane, A., Olde Keizer, R. A. C. M., Frederix, G., Vissers, L. E. L. M., De Boode, W. P., & Van Zelst-Stams, W. A. G. (2021). Congenital anomalies and genetic disorders in neonates and infants: a single-center observational cohort study. European Journal of Pediatrics, 181(1), 359–367. https://doi.org/10.1007/s00431-021-04213-w.
12. Vissers, L. E., Van Nimwegen, K., Schieving, J., Kamsteeg, E. J., Kleefstra, T., Yntema, H. G., Pfundt, R., Van Der Wilt, G. J., Brunner, H. G., Van Der Burg, S., Grutters, J. P., Veltman, J. A., & Willemsen, M. (2017). A clinical utility study of exome sequencing versus conventional genetic testing in Pediatric neurology. Genetics in Medicine, 19(9), 1055–1063. https://doi.org/10.1038/gim.2017.1.
13. Choi, M., Scholl, U. I., Ji, W., Liu, T., Tikhonova, I., Zumbo, P., Nayır, A., Bakkaloğlu, A., Özen, S., Sanjad, S. A., Nelson-Williams, C., Farhi, A., Mane, S., & Lifton, R. P. (2009). Genetic diagnosis by whole exome capture and massively parallel DNA sequencing. Proceedings of the National Academy of Sciences of the United States of America, 106(45), 19096–19101. https://doi.org/10.1073/pnas.0910672106.
14. Howell, K., Eggers, S., Dalziel, K., Riseley, J. R., Mandelstam, S., Myers, C. T., McMahon, J. M., Schneider, A., Carvill, G. L., Mefford, H. C., Scheffer, I. E., & Harvey, A. S. (2018). A population-based cost-effectiveness study of early genetic testing in severe epilepsies of infancy. Epilepsia, 59(6), 1177–1187. https://doi.org/10.1111/epi.14087.
15. Smith, H. S., Swint, J. M., Lalani, S. R., Yamal, J., De Oliveira Otto, M. C., Castellanos, S., Taylor, A., Lee, B., & Russell, H. V. (2019). Clinical Application of Genome and Exome Sequencing as a Diagnostic Tool for Pediatric Patients: a Scoping Review of the Literature. Genetics in Medicine, 21(1), 3–16. https://doi.org/10.1038/s41436-018-0024-6.
16. Nederlandse Zorgautoriteit (NZA, 2019). Tarieventabel DBC zorgproducten en overige producten. Available from: https://puc.overheid.nl/nza/doc/PUC_236092_22/1/.
17. Zorginstituut Nederland (ZIN, 2019). Kostenhandleiding: Methodologie van kostenonderzoek en

referentieprijzen voor economische evaluaties in de gezondheidszorg. Available from: https://www.zorginstituutnederland.nl/binaries/zinl/documenten/publicatie/2016/02/29/richtlijn-voor-het-uitvoeren-van-economische-evaluaties-in-de-gezondheidszorg/Richtlijn+voor+het+uitvoeren+van+economische+evaluaties+in+de+gezondheidszorg+%28verdiepingsmodules%29.pdf15-12

18. University Medical Center Groningen (UMCG, 2014). Tarieven 2014 voor verschillende vormen van genetisch onderzoek. Available from: https://www.umcg.nl/SiteCollectionDocuments/UMCG/Afdelingen/Genetica/Tarieven%20verrichtingen%20SEV%202014.pdf.
19. University Medical Center Groningen (UMCG, 2020). Tarieven Genetica- 2020. Available from: https://www.umcg.nl/SiteCollectionDocuments/UMCG/Afdelingen/Genetica/Tarieven%20Genetica%202020.pdf.
20. Nederlandse Zorgautoriteit (NZA, 2014). Zorgactiviteiten tabel toelichting. Available from: https://werkenmetdbcs.nza.nl/documenten-ziekenhuiszorg/overzicht-releases/dbc-pakket-2014/rz14a/tabellen-4/5352-zorgactiviteiten-tabel-toelichting-v20130926/file.
21. R Core Team, The R Project for Statistical Computing. Vienna, Austria: R foundation for Statistical Computing, Available from: http://www.Rproject.org.
22. Kuo, D. Z., Melguizo-Castro, M., Goudie, A., Nick, T. G., Robbins, J. M., & Casey, P. H. (2014). Variation in child health care utilization by medical complexity. Maternal and Child Health Journal, 19(1), 40–48. https://doi.org/10.1007/s10995-014-1493-0.
23. Dragojlovic, N., Van Karnebeek, C., Ghani, A., Genereaux, D., Kim, E., Birch, P., Adam, S., Du Souich, C., Elliott, A. M., Lehman, A., Lynd, L. D., Mwenifumbo, J., Nelson, T. N., Van Karnebeek, C., Friedman, J. M., & Lynd, L. D. (2020). The cost trajectory of the diagnostic care pathway for children with suspected genetic disorders. Genetics in Medicine, 22(2), 292–300. https://doi.org/10.1038/s41436-019-0635-6.
24. Corsten-Janssen, N., Bouman, K., Diphoorn, J. C. D., Scheper, A. J., Kinds, R., Mecky, J. E., Breet, H., Verheij, J., Suijkerbuijk, R. F., Duin, L. K., Manten, G. T. R., Van Langen, I. M., Sijmons, R. H., Sikkema-Raddatz, B., Westers, H., & Van Diemen, C. C. (2020). A prospective study on rapid exome sequencing as a diagnostic test for multiple congenital anomalies on fetal ultrasound. Prenatal Diagnosis, 40(10), 1300–1309. https://doi.org/10.1002/pd.5781.
25. Dempsey, E., Haworth, A., Ive, L., Dubis, R., Savage, H., Serra, E., Kenny, J., Elmslie, F., Greco, E., Thilaganathan, B., Mansour, S., Homfray, T., & Drury, S. (2021). A report on the impact of rapid prenatal exome sequencing on the clinical management of 52 ongoing pregnancies: a retrospective review. BJOG: An International Journal of Obstetrics and Gynaecology, 128(6), 1012–1019. https://doi.org/10.1111/1471-0528.16546.

SUPPLEMENTARY TABLES

Supplementary Table A. Overview of genetic diagnostic tests
Supplementary Table A can be found online at
https://doi.org/10.1016/j.ejmg.2022.104467

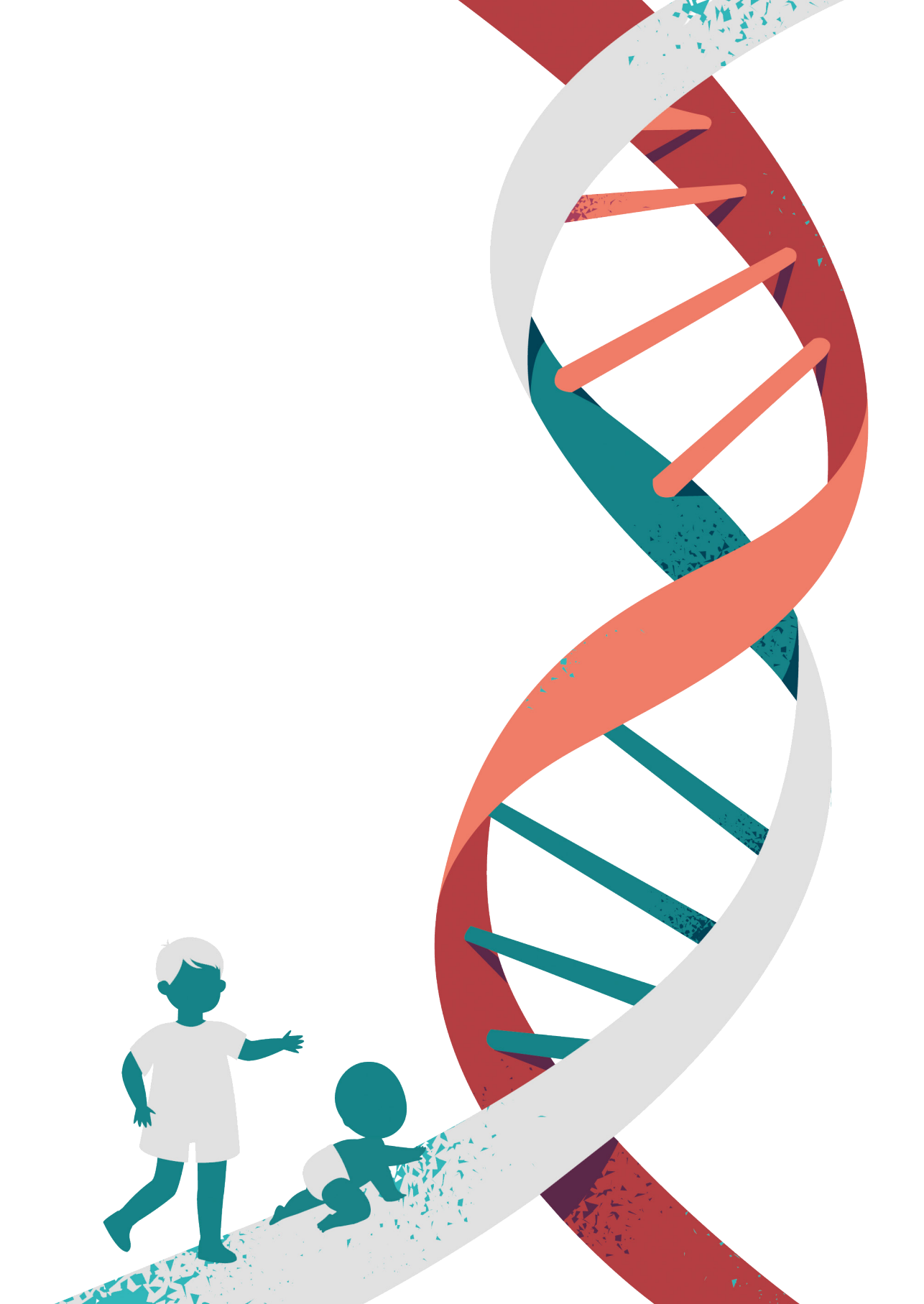
Supplementary Table B. Follow up time (days) per patient
Supplementary Table B can be found online at
https://doi.org/10.1016/j.ejmg.2022.104467

Supplementary Table C. Average costs per patient for scenario A, B, C and D implementing singleton-ES

	Current costs[a] (€)	Scenario A (€) Single-ES[b]	Scenario B (€) Single-ES[b]	Scenario C (€) Single-ES[b]	Scenario D (€) Single-ES[b]
Costs related to genetic testing					
Patients with multiple congenital anomalies (n = 136)	2,816	2,892	2,892	2,892	2,997
Patients with isolated congenital anomaly (n = 310)	954	2,892	2,892	0	2,997
Patients without congenital anomalies (n = 982)	208	2,892	0	0	0
Total (n = 1,428)	**619**	2,892 **(+367.2%)**	**903 (+45.9%)**	**275 (-55.5%)**	**936 (+51.2%)**
Overall healthcare costs					
Patients with multiple congenital anomalies (n = 136)	69,170	69,246	69,246	69,246	69,351
Patients with isolated congenital anomaly (n = 310)	33,414	35,352	35,352	32,460	35,457
Patients without congenital anomalies (n = 982)	18,382	21,065	18,173	18,173	18,173
Total (n = 1,428)	**26,482**	**28,755 (+8.6%)**	**26,767 (+1.1%)**	**26,139 (-1.3%)**	**26,799 (+1.2%)**

ES = exome sequencing; [a] Without taking into account the differences in follow-up time and excluding patients with a prenatal diagnosis; [b] Costs include pre-test genetic counseling, singleton-ES and post-test genetic counseling

Supplementary Table D. Genetic disorders, diagnostic tools, variants and detectability by ES
Supplementary Table D can be found online at
https://doi.org/10.1016/j.ejmg.2022.104467



CHAPTER 5

Rapid exome sequencing as first-tier test in neonates with suspected genetic disorder: results of a prospective multicenter clinical utility study in the Netherlands

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*Authors contributed equally; **Author list in Appendix 1

ABSTRACT

The introduction of rapid exome sequencing (ES) for critically ill neonates admitted to the neonatal intensive care unit has made it possible to impact clinical decision-making. Unbiased prospective studies to quantify the impact of rapid ES over routine genetic testing are, however, scarce. We performed a clinical utility study to compare rapid ES to conventional genetic diagnostic workup for critically ill neonates with suspected genetic disorders. In a prospective multicenter study involving five Dutch NICUs, we performed rapid ES in parallel to routine genetic testing for 60 neonates with a suspected genetic disorder, and monitored diagnostic yield and the time-to-diagnosis. To assess the economic impact of rapid ES, healthcare resource use was collected for all neonates. Questionnaires and Visual Analogue Scale (VAS) scores were obtained from parents and involved clinicians to gain insight into their perspectives of rapid ES. Rapid ES detected significantly more conclusive genetic diagnoses than routine genetic testing (20% vs. 10%, respectively, P<0.05), in a significantly shorter time-to-diagnosis (15 days (95% CI 10-20) vs. 59 days (95% CI 22-99)). Moreover, rapid ES reduced genetic diagnostic costs by 1.5% (€85 per neonate). Also, parents showed preference towards rapid ES, and involved clinicians reported clinical benefits for rapid ES. Our findings demonstrate the clinical utility of rapid ES for critically ill neonates based on increased diagnostic yield, shorter time-to-diagnosis and net healthcare savings. Together with the positive user experience, our observations warrant the widespread implementation of rapid ES as first-tier genetic test in critically ill neonates with disorders of suspected genetic origin.

5.1 INTRODUCTION

Genetic disorders are frequent causes of neonatal morbidity and mortality, and disease presentations are often undifferentiated at birth [1]. Since genetic disorders can progress fast, a rapid genetic diagnosis might provide the opportunity to reduce suffering, morbidity, and mortality, especially in critically ill infants and neonates [2, 3]. An essential indicator in genetic diagnostics is the turnaround time (TAT). Earlier research has demonstrated that rapid exome sequencing (ES) is related to a shortened TAT compared to conventional genetic diagnostic tests. The return of test results has decreased from several weeks or even months to a few days, thereby shortening the diagnostic odyssey and enabling precision medicine [4]. Genetic disorders and congenital anomalies (CA) affect around 6% of live births and are the leading reason for hospitalization in infants and neonates [5]. The presence of a genetic disorder can easily be missed because of the variable clinical presentation, often leading to a diagnostic odyssey requiring extensive evaluations, both clinically and genetically [6].

Approximately 2.5% of newborns is admitted to neonatal intensive care units (NICU) in the Netherlands [7]. The prevalence of genetic disorders is relatively high in critically ill newborns and this is accompanied by long hospitalization and high healthcare utilization [8]. Genetic testing of newborns using rapid ES at an earlier stage may reduce their diagnostic odyssey and enhance diagnosis-predicated precision. Early diagnosis may improve patient's clinical outcome and can potentially be life-saving [2]. Identifying a genetic diagnosis can also help to avoid ineffective (intensive) care in critically-ill newborns with poor prognosis.

Besides clinical implications, implementation of improved diagnostic tests can also have health economic impact. Gonzaludo et al. [9] have shown that children who are admitted to the hospital and diagnosed with a genetic disorder have a significant and disproportionate impact on resource use and related costs. By improving the diagnostic trajectory and shortening its length, future healthcare costs can be prevented for example by initiating adequate treatment earlier [10]. Before implementing a new technology, such as rapid ES, into diagnostic care, it is important to understand the possible financial/economic consequences. Since the costs of performing rapid ES have reduced over time, it is unknown what the actual costs will be when conventional genetic diagnostic trajectory is replaced by rapid ES.

Based on several studies showing that rapid ES provides a faster diagnosis, enabling timely precision medicine aiming to decrease morbidity and mortality of infants with genetic disorders [3, 5, 11-13], we hypothesized that rapid ES can positively affect diagnostic yield and the length of the diagnostic trajectory at lower costs. However, rapid

ES is still not sufficiently implemented in clinical guidelines of critically ill neonates as standard genetic care. Our group recently indicated that a prospective follow-up study is needed, in which current genetic diagnostic costs are compared to a parallel diagnostic trajectory in which rapid ES replaces all conventional genetic diagnostic testing [14]. Therefore, the aim of this study is to prospectively examine the clinical utility of rapid ES versus conventional genetic testing, by comparing clinical and economic outcomes.

5.2 METHODS

Study design

We performed a multicenter prospective parallel cohort study, in which we assessed the clinical utility of rapid ES compared to conventional genetic testing, i.e. routine genetic testing/the genetic trajectory based on decisions of the clinicians. In order to compare these two genetic trajectories, all study participants received both conventional genetic testing and rapid ES in parallel (Figure 1). This study design, in which the participants served as their own control, allowed the eliminate potential biases and confounders. Moreover, this approach allowed for direct comparison of both trajectories at three defined outcome measures including (i) genetic diagnostic yield, defined as the percentage of neonates receiving a conclusive genetic diagnosis, (ii) time-to-diagnosis (TTD), calculated as the time between the request of the first genetic test and receiving the conclusive genetic test results, and (iii) costs associated with (genetic) health care resource use in the first 2 years of life.

This study was approved by the Medical Research Ethics Committee Arnhem/Nijmegen under file number 2016–2486/ NL57511.091.16.

Patient recruitment

We studied 60 neonates admitted to a NICU in five out of ten centers in the Netherlands between May 2017-January 2019. This sample size was calculated based on a two-sided chi-square test, using a power of 80% and a significance level of 0.05. The diagnostic yield of routine genetic testing was estimated to be 5% and 25% for rapid ES. The sample size calculating resulted in a minimum of 55 patients, with a significance level of <0.001. Taking into account a possible drop-out of 10%, during the course of the study, 60 patients were recruited. Criteria for inclusion were a post-natal age less than 3 months at presentation, and high suspicion of a genetic disorder (as assessed by neonatologist and/or clinical geneticist based on the neonate's clinical presentation). In addition, EDTA blood samples of both biological parents were required for participation. Exclusion criteria were a previously (prenatally) established genetic diagnosis, or a clinically phenotype highly associated with trisomy 13, 18, 21, or monosomy X. Parents

were informed about the study by the attending clinician, in consultation with a clinical geneticist, if routine genetic testing was indicated. Written informed consent was obtained for all participating families.

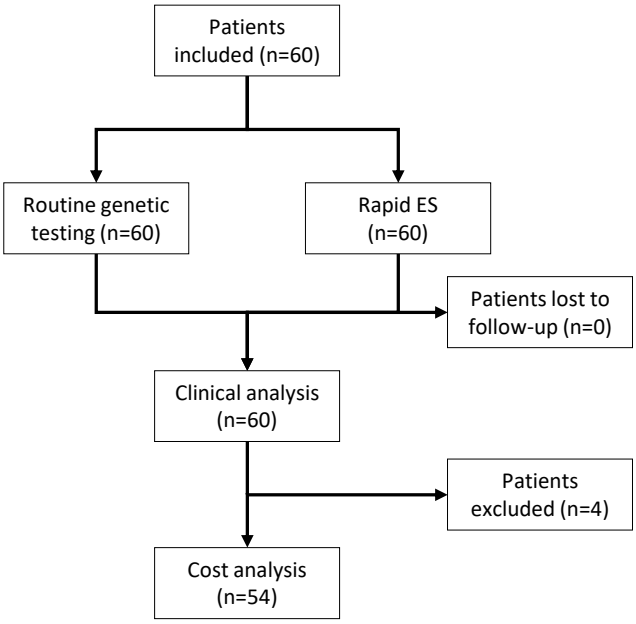


Figure 1. Flowchart of the included patients. Overview of patients enrolled in this study, including patients lost to follow-up or excluded.
ES = exome sequencing.

Clinical description to assess representativeness of cohort

Clinical features were scored using the human phenotype ontology (HPO) terms (Supplementary Table 1) [15]. CA were considered isolated when affecting a single organ system, and as multiple congenital anomalies (MCA) when affecting two or more organ systems. Representativeness of the cohort was determined by comparison to our recent analysis of a retrospective cohort of >1,400 neonates admitted to the NICU for which we described routine genetic care, uptake of genetic testing and diagnostic potential, in addition to economic models predicting effects of the use of rapid ES in a NICU setting [14, 16].

Rapid ES procedure

The rapid ES procedure was performed in the ISO15189 accredited genetic diagnostic laboratories affiliated to the five NICUs. Whereas minor technical differences exist

between centers, such as for instance different enrichment kits or sequencing equipment used, the overall procedures were similar [17-19]. Importantly, data interpretation was in all neonates guided by the clinical referral, and could consist of interpretation of disease-gene specific panels, interpretation of the Mendeliome (all genes with confirmed OMIM-disease-gene associations), interpretation of all genetic variants to allow discovery of novel candidate disease genes (open exome strategy), or a combination of these strategies. Variants were clinically interpreted based on a 5-class system, with class 1/2 representing (likely) benign variants, class 4/5 representing (likely) pathogenic variants, and class 3 representing variants of unknown clinical significance [20].

Diagnostic yield

For each neonate, we monitored the routine genetic diagnostic trajectory (Supplementary Table 2). In routine care, the type of genetic tests and the number of tests were left at the discretion of the clinical geneticist. In parallel, rapid ES was performed as study intervention. For neonates where ES was requested as part of routine diagnostic care, the ES was not performed in duplicate, but the results of the rapid ES were used as such. A conclusive diagnosis was defined as a laboratory-confirmed genetic diagnosis based on the identification of a (likely) pathogenic (class 4 and 5) variant in concordance with the patients' phenotype. Variants of unknown clinical significance (class 3) in a known disease gene in concordance with the neonates' phenotype were considered a possible diagnosis. For comparison of the diagnostic yield, only conclusive diagnoses were considered.

Turnaround time (TAT) and time-to-diagnosis (TTD)

To gain insight into the time spent to obtain a genetic diagnosis, we discriminated between the TAT, and the TTD. The TAT could be assessed for all tests and describes the time between receipt of the diagnostic sample and the final test report, irrespective of the obtained result (e.g. diagnosis or no diagnosis). In contrast, the TTD was only determined for neonates who received a conclusive diagnosis. The latter was chosen as this reflects an objective end point of genetic diagnostic care for index patients. The TTD was measured from the moment the first genetic test was requested by the involved clinician until the return of the final conclusive genetic diagnostic report. Of note, in case ES was request in the conventional diagnostic strategy, only the rapid ES was performed, and the TAT of routine ES was censored to overcome the need to perform the ES procedure twice. To determine this censored TAT of ES in conventional diagnostic testing, the TAT of ES was determined from a random, anonymized, set of individuals, unrelated to this project, but equal in size of the number of neonates receiving rapid ES. This resulted in a TAT for ES of 105 days (95% CI 96-113 days).

Costs of genetic diagnostic trajectory

Health care consumption was collected from the electronic patient file of 56/60 patients.

For four neonates, these data could not be collected and therefore these patients were excluded from the costs analyses. Healthcare activities were linked to their unit prices (index year 2020) retrieved from the Dutch Healthcare Authority (Nederlandse Zorgautoriteit; NZA) and National Healthcare Institute (Zorginstituut Nederland; ZIN) [21, 22], after which a distinction was made between costs related to genetic diagnostic testing and other health care related costs.

For all healthcare data, average costs per neonate were calculated, including the minimum, maximum and median. Costs were divided into seven categories based on their type of healthcare activity: diagnostics, hospitalization, consult, surgery, medicines, genetic and other healthcare costs. Per category, a percentage of total healthcare costs was calculated. For the category 'genetic costs', the types of genetic tests performed were also retrieved to provide more detailed insight to the build-up of costs associated with genetic testing.

Next, two cost analyses were performed, firstly the economic impact of implementation of rapid ES compared to the conventional genetic testing; and secondly, the evaluation of the timing of expenditure for genetic testing, differentiating between costs made during the neonatal period (first 28 days starting at birth) and costs during the post-neonatal period with a maximum follow-up period of two years after birth.

Statistical methods

Data analysis of the clinical variables was performed in Excel (version 2016) and the cost analysis was performed in R (version 4.0.3) [23]. In more detail, normal distributed data were expressed in mean and standard deviation; Median and interquartile ranges were used in data with a skewed distribution. Paired samples t-test was performed to analyze whether the difference in TAT and costs between the two diagnostic trajectories were significant.

5.3 RESULTS

Representativeness of cohort

A total of 60 newborns admitted to the NICU with clinical features suggestive for a possible genetic disorder were enrolled in this study. Demographic information is presented in Table 1, with detailed clinical characteristics per patient summarized in Supplementary Table 1. When comparing the cohort to a recently published, retrospectively collected cohort of NICU patients [14], we observe a shift towards more newborns with CA (78% vs. 32%, $p<0.001$), as expected given our study purpose and the relation between genetic disorders and CA [14]. Patients with CA are more frequently genetic tested than

neonates without. Within the subgroup of neonates with CA, neonates more often showed multiple CA (70%) than isolated anomalies (30%).

Table 1. Clinical characteristics

	N= 60
Male/Female	32 (53%) / 28 (47%)
Gestational age	
Extremely preterm (< 28 weeks)	7 (12%)
Very preterm (28 weeks – 33 weeks)	15 (25%)
Preterm (34 weeks – 36 weeks)	8 (13%)
Term (37 weeks – 41 weeks)	29 (48%)
Post-term (>42 weeks)	1 (2%)
Clinical features	
Congenital anomalies	47 (78%)
• Isolated	14/47 (25%)
• Multiple	33/47 (53%)
No congenital anomaly	13 (22%)
Prenatal	
Prenatal ultrasound abnormalities	30 (50%)

Comparison of diagnostic yield, TAT, and TTD in routine genetic testing versus rapid ES

For all 60 neonates, the genetic diagnostic trajectory started in the neonatal period, at an average age of 8 days (95% CI 6-10 days). From this start point onwards, the neonates received both routine genetic testing and rapid ES in parallel, allowing to directly compare outcome measures as neonates served as their own controls.

In routine genetic testing, a total of 112 genetic tests were performed, resulting in an average of 1.87 tests per patient (range 1-5; Supplementary Figure 1; Supplementary Table 2). In total, 7 different types of assays were requested, with genomic microarray (46/112; 41%) and routine ES (44/112; 39%) being most frequently ordered. In 6 out of 60 neonates (10%) a conclusive genetic diagnosis could be identified (Table 2). The mean TAT of the routine genetic diagnostic trajectory by tests initiated in the neonatal period was 81 days (95% CI 71-92), with the main driver of this relatively long TAT being routine ES (Supplementary Table 2). For patients with a conclusive diagnosis, the average TTD was 59 days (95% CI 23-98).

Parallel to the conventional genetic diagnostic trajectory, all neonates received trio-based rapid ES. These efforts resulted in conclusive genetic diagnosis in 12 of 60 neonates

(20%), providing higher diagnostic yield than the routine genetic tests (Table 2). Rapid ES had an average TAT of 12 days (95% CI 10-14 days), being significantly shorter than the average 81-day TAT in routine genetic testing (p<0.001; Supplementary Table 2). The average TTD was 15 days (95% CI 10-20) for patients with a conclusive genetic diagnosis, which is four times faster than for routine genetic testing (59 days).

Table 2. Genetically confirmed diagnosis and diagnostic methods

		Rapid ES		
		Conclusive diagnosis	**No diagnosis**	**Total**
Conventional genetic testing	**Conclusive diagnosis**	**6** - 22q11 deletion syndrome(2x) - Mowat-Wilson syndrome - Renal cysts and diabetes syndrome - Trisomy 21 - Turner syndrome[a]	**0**	**6**
	No diagnosis	**6** - Costello syndrome - Developmental and epileptic encephalopathy - Noonan syndrome (3x) - X-linked myotubular myopathy	**48** *undiagnosed patients*	**54**
	Total	**12**	**48**	

ES = exome sequencing; [a] In this case, rapid ES detected a possible 45,X0 based on variant characteristics on the X-chromosome, the conventional genetic test, performed in parallel to rapid ES, confirmed a 45,X0 chromosome profile.

Concordance between genetic diagnostic trajectories

In our cohort of 60 neonates, 12 conclusive genetic diagnoses were obtained (Supplementary Table 3). 8 of the 12 (66%) diagnoses were based on (de novo) single nucleotide variants (SNVs), known to lead to monogenic disorders with high genetic and clinical heterogeneity. In two neonates (17%), an aneuploidy was detected including trisomy 21 resulting in Down´s syndrome, and 45,X0 causing Turner syndrome without a typical phenotype. The remaining two diagnoses (17%) were based on smaller copy number variants (CNVs). Of these 12 diagnoses, 6 were detected by both genetic diagnostic trajectories, albeit that for one of these, an additional confirmatory test was needed in the rapid ES trajectory. The remaining six diagnoses were only obtained in the genetic trajectory using rapid ES (Table 2).

Comparison of costs of genetic diagnostic trajectories

In the routine genetic care pathway, the average total healthcare costs during the first two years of life were €125,826 per neonate (Table 3), with 5.2% of the expenditures (on average €6,568 per neonate) for genetic care. Of the expenditure for genetic care,

83.6% (on average €5,494 per neonate) corresponded to routine genetic diagnostic testing, and 16.4% (on average €1,074 per neonate) was spent on genetic consultation.

Table 3. Average costs per patient

	Average per patient (€)	Min (€)	Median (€)	Max (€)	Percentage
Diagnostics	16,648	234	8,310	101,206	13.2%
Hospitalization	97,552	9,038	53,273	318,838	77.5%
Consult	2,725	0	1,606	14,658	2.2%
Surgery	519	0	0	11,045	0.5%
Genetics	6,568	392	6,882	14,964	5.2%
Medicines	519	0	0	22,608	0.4%
Other	1,223	0	179	12,205	1.0%
Total	125,826	10,273	69,224	416,473	

To assess the overall impact rapid ES as replacement for the routine genetic testing, costs of routine genetic testing (€5,494 per neonate) were substituted by the costs of trio-based rapid ES (€5,409 per neonate; Table 4), resulting in a (non-significant, p=0.23) reduction in genetic costs by 1.5% (i.e. €85 per neonate). We subsequently aimed on gaining insight into the timing of healthcare utilization and associated costs (Figure 2). Of the €5,494 for genetic diagnostic testing, €4,781 (87%) was spent in the neonatal period (Figure 2).

Table 4. Average number of declarations and associated costs per neonate

		Average number of declarations per neonate	
Genetic diagnostic test	**Unit price (€)**	**Routine genetic testing[c]**	**Rapid ES**
Karyotype	933	0.23	-
FISH	818	0.05	-
Genomic microarray	825	1.09	-
Sanger sequencing	561	0.13	-
Disease specific gene	871	0.05	-
Gene panel	1,768	0.04	-
ES[a]	5,409	0.73	1.00
Other[b]	640	0.30	-
Total		**2.62 (€5,494)**	**1.00 (€5,409)**

[a] Trio-ES, so the unit price of singleton ES (€1,803) was multiplied by three; [b] Follow-up genetic test in one or more genes; [c] Of note, the number of average tests performed in routine care on the index case (i.e. 1.87) deviates from the average number of total declarations as also parental samples are tested, and result in healthcare related costs to obtained diagnosis. FISH = fluorescence in situ hybridization; ES = exome sequencing.

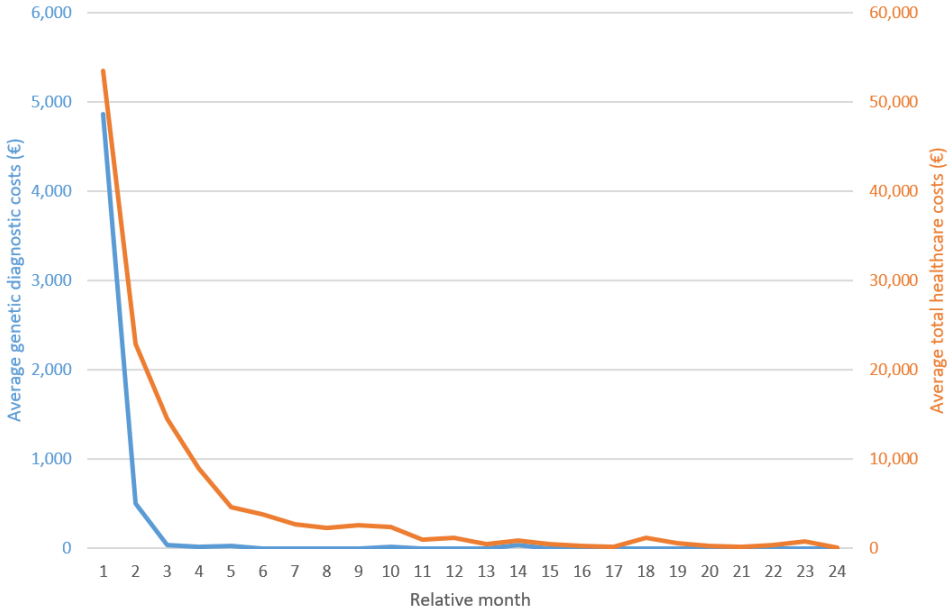


Figure 2. Average genetic costs per month per patient over time. Overview of average genetic diagnostic costs (blue) and average total healthcare costs (orange) over time, starting from birth (relative month). Costs decrease over time and 88% of the average genetic diagnostic costs are made during the neonatal period.

5.4 DISCUSSION

We studied the genetic diagnostic trajectory of ill newborns with suspected genetic disease to evaluate the clinical utility of rapid ES in comparison with conventional genetic diagnostic testing in a multicenter prospective observational study at tertiary NICUs throughout the Netherlands. We demonstrate that the use of rapid ES in neonates with a suspected genetic disorder, is associated with a higher diagnostic yield and leads to a faster diagnosis, without an increase in costs.

This study showed that rapid ES had a diagnostic yield of 20%. This diagnostic rate was lower compared to other studies yielding 36-57% in neonatal settings [24, 25]. This might be explained by our clinical preselection that was aimed to represent all neonates admitted with a disorder suspected of a genetic origin. This inclusion criteria in this study were less strict compared to previous studies. Besides, the make-up of the group of newborns for this study can be influenced by the difference in genetic expertise of the clinicians involved in the selection namely pediatricians and not clinical geneticists. In addition, we excluded neonates who already received prenatal positive rapid ES testing. With prenatal rapid ES having found its place in routine testing upon ultrasound

abnormalities in the same time as when this clinical utility study was performed, it is also within reason to expect that this has influenced the overall diagnostic yield in this cohort [17]. Nonetheless, given the broad spectrum of diseases studied, rapid ES has been shown to be a suitable genetic diagnostic tool for neonates suspected of a genetic disorder in different disease areas. Although some genetic diseases exhibit themselves within the first 28 days of life or shortly thereafter, some clinical symptoms may be undifferentiated, especially in the early days of life [26]. We found that the presence of MCA increased the likelihood of finding a conclusive genetic diagnosis.

Identifying the genetic cause of disease may lead to patient tailored clinical management, and facilitate shared decision-making regarding end-of-life decisions, either way reducing ineffective, empirical, or detrimental therapies [27]. As an example, in one of the included patients with a severe congenital heart defect, epilepsy and agenesis of corpus callosum, the diagnosis Mowat-Wilson syndrome was established. Importantly, a former study of our group, also demonstrating a high diagnostic rate for MCA [14]. This diagnosis was only identified by the rapid ES trajectory, and helped the parents and the involved clinician in their decision about optimal care. This prevented ineffective cardiorespiratory support given the severe prognosis related to the underlying genetic disorder and withdrawal of intensive treatment was initiated.

Based on our recent study in which we modelled scenarios for the implementation of rapid ES for neonates admitted to the NICU based on a retrospective cohort of >1,400 neonates, it was expected that the use of rapid ES would lead to an increase of genetic diagnostic costs [14, 16]. However, establishing a genetic diagnosis early in life would also prevent a diagnostic odyssey, and was considered the main incentive, along with potential future saving on futile testing, for the use of rapid ES in critically ill neonates admitted at the NICU [27, 28]. Our prospective evaluation however shows that, in addition to an increased diagnostic yield and a shorter TTD, rapid ES leads to reduced costs related to genetic testing. A likely explanation for this difference in anticipated increase, and observed decrease in costs, is identified by the uptake of ES as part of the routine genetic diagnostic trajectory (e.g. with an average TAT of 81 days). Results of this study showed that ES is implemented during the routine genetic diagnostic trajectory, which caused a larger increase in costs compared to the genetic trajectory in which only rapid ES was performed. In our retrospective analysis, ES was only rarely used. Prospectively, we now noticed that ES was requested in 73% (44/60) of the cohort, despite the realization of neonatologists and clinical geneticist that the TAT would be too long to impact decision-making while at the NICU; the prevention of a diagnostic odyssey prevailed. Our study now objectively shows that rapid ES, as such, is not increasing costs compared to current use of genetic diagnostics, and highlights that systematic use of rapid ES for all neonates with (M)CA, admitted at the NICU, would increase diagnostic

yield. Interestingly, our longitudinal data of two-year follow-up showed that there is also limited uptake of additional genetic testing after the initial rapid ES, and >95% of genetic diagnostic costs concentrated in the neonatal period. However, costs associated with (periodic) re-analysis of the existing rapid ES data for neonates without a genetic diagnosis are still to be expected. To what extent these additional costs outweigh costs saving elsewhere in the care path of these patients, is still to be determined. However, based on earlier research, it is assumed that shortening of the diagnostic trajectory by implementing rapid ES will lead to a reduction of healthcare costs, also taking into account cost savings in the future [29-31]. Moreover, withdrawal of care after confirmation of a genetic diagnosis associated with a very poor prognosis will prevent superfluous medical expenses.

Proper genetic consultation of parents of newborns is essential for well-informed decision-making and to prevent harm or decisional regret afterwards [32]. Prior to the start of our study several patient associations were hesitant about implementing rapid ES in neonates admitted to the NICU because of the potential extra (emotional) burden it may bring to parents of neonates. We performed an explorative study about the (emotional) burden of parents and clinicians (neonatologists), showing that most of the parents experienced use of rapid ES not as stressful and evaluated it as having a positive impact on clinical decision-making (Supplementary Figure 2). Dedicated studies with validated questionnaires are, however, needed to substantiate firm conclusions in end-user perspectives.

A limitation of our study is the relatively small sample size per center, hampering the analysis on representativeness of the cases per center. However, our approach did allow us to more broadly assess feasibility of rapid ES implementation since multiple centers participated in this study. Furthermore, there is no significant difference between the included centers regarding clinical decision-making. Therefore, we expect no limitations regarding generalizability of the results. As these NICUs are also referral centers with a high volume of transfers and retro-transfers, it is difficult to capture an infant's entire diagnostic odyssey, particularly if it began in another institution. However, our study is valuable in reflecting current practices at NICUs that cares for many neonates with rare and likely genetic disorders.

Overall, it can be concluded that rapid ES as a first-tier genetic test in the NICU is clinically relevant and beneficial for newborns, their parents and treating clinicians. Implementation of rapid ES will increase diagnostic yield and provides a diagnosis more rapidly than conventional genetic testing, without incurring higher costs to the healthcare system enabling individualized clinical management.

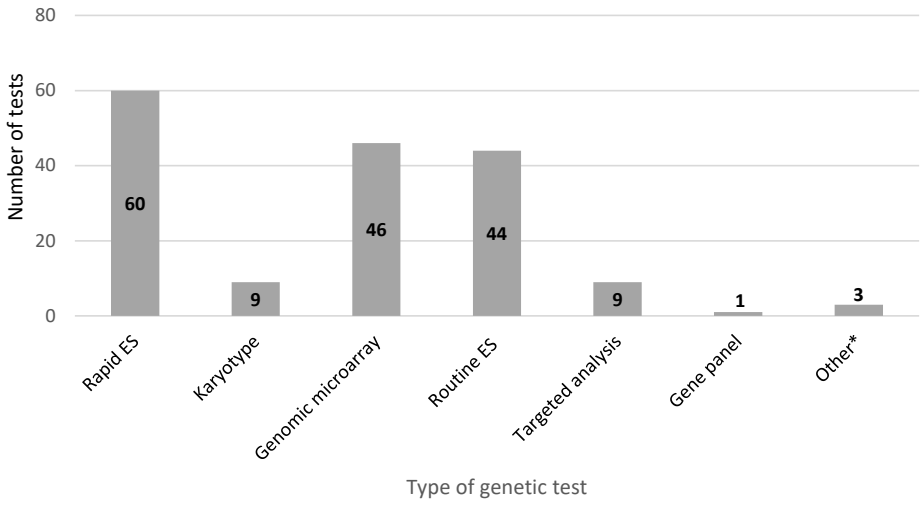
REFERENCES

1. Saunders, C. J., Miller, N., Soden, S. E., Dinwiddie, D. L., Noll, A., Alnadi, N. A., Andraws, N., Patterson, M., Krivohlavek, L. A., Fellis, J., Humphray, S., Saffrey, P., Kingsbury, Z., Weir, J. C., Betley, J. R., Grocock, R., Margulies, E. H., Farrow, E., Artman, M., . . . Kingsmore, S. F. (2012). Rapid Whole-Genome sequencing for genetic disease diagnosis in neonatal intensive care units. Science Translational Medicine, 4(154). https://doi.org/10.1126/scitranslmed.3004041.
2. Chung, C. C. Y., Leung, G. K. C., Mak, C. C. Y., Fung, J. L. F., Lee, M., Pei, S. L. C., Yu, M. H., Hui, V. C. C., Chan, J. C. K., Chau, J. F. T., Chan, M. C., Tsang, M. H., Wong, W. H. S., Tung, J. Y., Lun, K., Ng, Y. K., Fung, C. W., Wong, M. S. C., Wong, R., . . . Chung, B. H. (2020). Rapid whole-exome sequencing facilitates precision medicine in paediatric rare disease patients and reduces healthcare costs. The Lancet Regional Health-Western Pacific, 1, 100001. https://doi.org/10.1016/j.lanwpc.2020.100001.
3. Lunke, S., Eggers, S., Wilson, M., Patel, C., Barnett, C., Pinner, J., Sandaradura, S. A., Buckley, M. F., Krzesinski, E. I., De Silva, M. G., Brett, G. R., Boggs, K., Mowat, D., Kirk, E. P., Adès, L. C., Akesson, L. S., Amor, D. J., Ayres, S., Baxendale, A., . . . Stark, Z. (2020). Feasibility of Ultra-Rapid exome sequencing in critically ill infants and children with suspected monogenic conditions in the Australian public health care system. JAMA, 323(24), 2503. https://doi.org/10.1001/jama.2020.7671.
4. Smith, L. D., Willig, L. K., & Kingsmore, S. F. (2015). Whole-Exome sequencing and Whole-Genome sequencing in critically ill neonates suspected to have Single-Gene disorders. Cold Spring Harbor Perspectives in Medicine, 6(2), a023168. https://doi.org/10.1101/cshperspect.a023168.
5. Farnaes, L., Hildreth, A., Sweeney, N. M., Clark, M. M., Chowdhury, S., Nahas, S., Cakici, J. A., Benson, W., Kaplan, R. H., Kronick, R., Bainbridge, M. N., Friedman, J., Gold, J. J., Ding, Y., Veeraraghavan, N., Dimmock, D., & Kingsmore, S. F. (2018). Rapid whole-genome sequencing decreases infant morbidity and cost of hospitalization. Npj Genomic Medicine, 3(1). https://doi.org/10.1038/s41525-018-0049-4.
6. Swaggart, K. A., Swarr, D. T., Tolusso, L., He, H., Dawson, D. B., & Suhrie, K. (2019). Making a genetic diagnosis in a Level IV neonatal Intensive care unit population: who, when, how, and at what cost? The Journal of Pediatrics, 213, 211-217.e4. https://doi.org/10.1016/j.jpeds.2019.05.054.
7. Perined. Utrecht (2022). Available from: https://www.peristat.nl.
8. Smith, H. S., Swint, J. M., Lalani, S. R., De Oliveira Otto, M. C., Yamal, J., Russell, H. V., & Lee, B. (2020). Exome sequencing compared with standard genetic tests for critically ill infants with suspected genetic conditions. Genetics in Medicine, 22(8), 1303–1310. https://doi.org/10.1038/s41436-020-0798-1.
9. Gonzaludo, N., Belmont, J. W., Gainullin, V. G., & Taft, R. J. (2019). Estimating the burden and economic impact of pediatric genetic disease. Genetics in Medicine, 21(8), 1781–1789. https://doi.org/10.1038/s41436-018-0398-5.
10. Choi, M., Scholl, U. I., Ji, W., Liu, T., Tikhonova, I., Zumbo, P., Nayır, A., Bakkaloğlu, A., Özen, S., Sanjad, S. A., Nelson-Williams, C., Farhi, A., Mane, S., & Lifton, R. P. (2009). Genetic diagnosis by whole exome capture and massively parallel DNA sequencing. Proceedings of the National Academy of Sciences of the United States of America, 106(45), 19096–19101. https://doi.org/10.1073/pnas.0910672106.
11. Gubbels, C. S., VanNoy, G. E., Madden, J. A., Copenheaver, D., Yang, S., Wojcik, M. H., Gold, N. B., Genetti, C. A., Stoler, J. M., Parad, R. B., Rumyantsev, S. A., Bodamer, O. A., Beggs, A. H., Juusola, J., Agrawal, P. B., & Yu, T. W. (2020). Prospective, phenotype-driven selection of critically ill neonates for rapid exome sequencing is associated with high diagnostic yield. Genetics in Medicine, 22(4), 736–744. https://doi.org/10.1038/s41436-019-0708-6.
12. Wojcik, M. H., Schwartz, T. S., Yamin, I., Edward, H. L., Genetti, C. A., Towne, M. C., & Agrawal, P. B. (2018). Genetic disorders and mortality in infancy and early childhood: delayed diagnoses and missed opportunities. Genetics in Medicine, 20(11), 1396–1404. https://doi.org/10.1038/gim.2018.17.
13. Śmigiel, R., Biela, M., Szmyd, K., Błoch, M., Szmida, E., Skiba, P., Walczak, A., Gasperowicz, P., Kosińska, J., Rydzanicz, M., Stawiński, P., Biernacka, A., Zielińska, M., Gołębiowski, W., Jalowska, A., Ohia, G., Głowska, B., Walas, W., Królak-Olejnik, B., . . . Płoski, R. (2020). Rapid Whole-Exome sequencing as a diagnostic tool in a Neonatal/Pediatric Intensive Care unit. Journal of Clinical Medicine, 9(7), 2220. https://doi.org/10.3390/jcm9072220.
14. Marouane, A., Olde Keizer, R. A. C. M., Frederix, G., Vissers, L. E. L. M., De Boode, W. P., & Van Zelst-Stams, W. A. G. (2021). Congenital anomalies and genetic disorders in neonates and infants: a single-center observational cohort study. European Journal of Pediatrics, 181(1), 359–367. https://doi.org/10.1007/

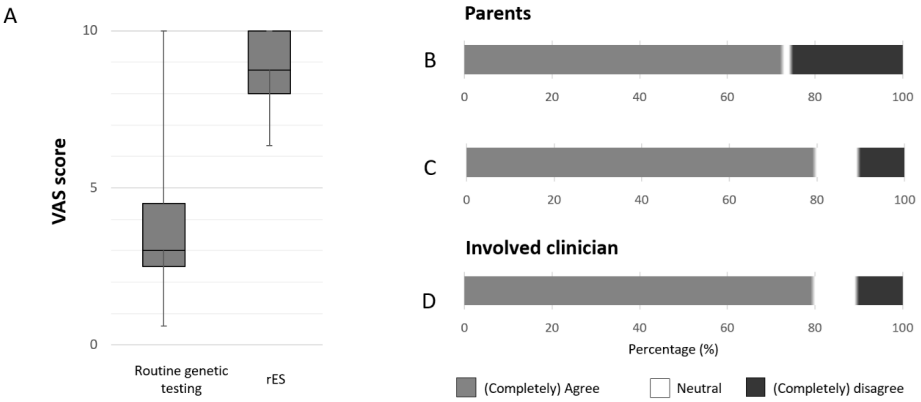
s00431-021-04213-w.
15. Köhler, S., Vasilevsky, N., Engelstad, M., Foster, E. D., McMurry, J. A., Aymé, S., Baynam, G., Bello, S. M., Boerkoel, C. F., Boycott, K. M., Brudno, M., Buske, O. J., Chinnery, P. F., Cipriani, V., Connell, L. E., Dawkins, H., DeMare, L. E., Devereau, A., De Vries, B. B., . . . Robinson, P. N. (2016). The Human Phenotype Ontology in 2017. Nucleic Acids Research, 45(D1), D865–D876. https://doi.org/10.1093/nar/gkw1039.
16. Olde Keizer, R. A. C. M., Marouane, A., Deden, A. C., Van Zelst-Stams, W. A. G., De Boode, W. P., Keusters, W. R., Henneman, L., Van Amstel, J. K. P., Frederix, G. W., & Vissers, L. E. L. M. (2022). Medical costs of children admitted to the neonatal intensive care unit: The role and possible economic impact of WES in early diagnosis. European Journal of Medical Genetics, 65(5), 104467. https://doi.org/10.1016/j.ejmg.2022.104467.
17. Deden, C., Neveling, K., Zafeiropopoulou, D., Gilissen, C., Pfundt, R., Rinne, T., De Leeuw, N., Faas, B. H. W., Gardeitchik, T., Sallevelt, S. C. E. H., Paulussen, A. D. C., Stevens, S. J., Sikkel, E., Elting, M. W., Van Maarle, M. C., Diderich, K. E. M., Corsten-Janssen, N., Lichtenbelt, K. D., Lachmeijer, G., . . . Van Zelst-Stams, W. A. G. (2020). Rapid whole exome sequencing in pregnancies to identify the underlying genetic cause in fetuses with congenital anomalies detected by ultrasound imaging. Prenatal Diagnosis, 40(8), 972–983. https://doi.org/10.1002/pd.5717.
18. Imafidon, M. E., Sikkema-Raddatz, B., Abbott, K. M., Meems-Veldhuis, M. T., Swertz, M. A., Van Der Velde, K. J., Beunders, G., Bos, D. K., Knoers, N. V. A. M., Kerstjens-Frederikse, W. S., & Van Diemen, C. C. (2021). Strategies in rapid genetic diagnostics of critically Ill children: Experiences from a Dutch University hospital. Frontiers in Pediatrics, 9. https://doi.org/10.3389/fped.2021.600556.
19. Monroe, G. R., Frederix, G. W., Savelberg, S. M. C., De Vries, T. I., Duran, K., Van Der Smagt, J. J., Terhal, P. A., Van Hasselt, P. M., Kroes, H. Y., Verhoeven-Duif, N. M., Nijman, I. J., Carbo, E. C., Van Gassen, K., Knoers, N. V. A. M., Hövels, A. M., Van Haelst, M. M., Visser, G., & Van Haaften, G. (2016). Effectiveness of whole-exome sequencing and costs of the traditional diagnostic trajectory in children with intellectual disability. Genetics in Medicine, 18(9), 949–956. https://doi.org/10.1038/gim.2015.200.
20. Richards, S., Aziz, N., Bale, S. J., Bick, D., Das, S., Gastier-Foster, J. M., Grody, W. W., Hegde, M., Lyon, E., Spector, E., Voelkerding, K. V., & Rehm, H. L. (2015). Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genetics in Medicine, 17(5), 405–424. https://doi.org/10.1038/gim.2015.30.
21. Nederlandse Zorgautoriteit (NZA, 2014). Zorgactiviteiten tabel toelichting. Available from: https://werkenmetdbcs.nza.nl/documenten-ziekenhuiszorg/overzicht-releases/dbc-pakket-2014/rz14a/tabellen-4/5352-zorgactiviteiten-tabel-toelichting-v20130926/file.
22. Zorginstituut Nederland (ZIN, 2019). Kostenhandleiding: Methodologie van kostenonderzoek en referentieprijzen voor economische evaluaties in de gezondheidszorg. Available from: https://www.zorginstituutnederland.nl/binaries/zinl/documenten/publicatie/2016/02/29/richtlijn-voor-het-uitvoeren-van-economische-evaluaties-in-de-gezondheidszorg/Richtlijn+voor+het+uitvoeren+van+economische+evaluaties+in+de+gezondheidszorg+%28verdiepingsmodules%29.pdf15-12
23. R Core Team, The R Project for Statistical Computing. Vienna, Austria: R foundation for Statistical Computing, Available from: http://www.Rproject.org.
24. Kapil, S., Fishler, K. P., Euteneuer, J. C., & Brunelli, L. (2018). Many newborns in level IV NICUs are eligible for rapid DNA sequencing. American Journal of Medical Genetics- Part A, 179(2), 280–284. https://doi.org/10.1002/ajmg.a.61011.
25. Stark, Z., Tan, T. Y., Chong, B., Brett, G. R., Yap, P., Walsh, M., Yeung, A., Peters, H., Mordaunt, D., Cowie, S., Amor, D. J., Savarirayan, R., McGillivray, G., Downie, L., Ekert, P. G., Theda, C., James, P., Yaplito-Lee, J., Ryan, M. M., . . . White, S. (2016). A prospective evaluation of whole-exome sequencing as a first-tier molecular test in infants with suspected monogenic disorders. Genetics in Medicine, 18(11), 1090–1096. https://doi.org/10.1038/gim.2016.1.
26. French, C., Delon, I., Dolling, H., Sanchis-Juan, A., Shamardina, O., Mégy, K., Abbs, S., Austin, T., Bowdin, S., Branco, R. G., Firth, H. V., Children, N. G., Rowitch, D. H., & Raymond, F. L. (2019). Whole genome sequencing reveals that genetic conditions are frequent in intensively ill children. Intensive Care Medicine, 45(5), 627–636. https://doi.org/10.1007/s00134-019-05552-x.
27. Krantz, I. D., Medne, L., Weatherly, J., Wild, K. T., Biswas, S., Devkota, B., Hartman, T. R., Brunelli, L., Fishler, K. P., Abdul-Rahman, O., Euteneuer, J. C., Hoover, D. M., Dimmock, D., Cleary, J. P., Farnaes, L., Knight, J., Schwarz, A., Vargas-Shiraishi, O., Wigby, K., . . . Taft, R. J. (2021). Effect of Whole-Genome sequencing on

the clinical management of acutely ill infants with suspected genetic disease. JAMA Pediatrics, 175(12), 1218. https://doi.org/10.1001/jamapediatrics.2021.3496.
28. Dimmock, D., Caylor, S., Waldman, B., Benson, W., Ashburner, C., Carmichael, J., Carroll, J., Cham, E., Chowdhury, S., Cleary, J. P., D'Harlingue, A. E., Doshi, A., Ellsworth, K. A., Galarreta, C. I., Hobbs, C. A., Houtchens, K. A., Hunt, J. N., Joe, P., Joseph, M., . . . Farnaes, L. (2021). Project Baby Bear: Rapid precision care incorporating rWGS in 5 California children's hospitals demonstrates improved clinical outcomes and reduced costs of care. American Journal of Human Genetics, 108(7), 1231–1238. https://doi.org/10.1016/j.ajhg.2021.05.008.
29. Howell, K., Eggers, S., Dalziel, K., Riseley, J. R., Mandelstam, S., Myers, C. T., McMahon, J. M., Schneider, A., Carvill, G. L., Mefford, H. C., Scheffer, I. E., & Harvey, A. S. (2018). A population-based cost-effectiveness study of early genetic testing in severe epilepsies of infancy. Epilepsia, 59(6), 1177–1187. https://doi.org/10.1111/epi.14087.
30. Vissers, L. E., Van Nimwegen, K., Schieving, J., Kamsteeg, E. J., Kleefstra, T., Yntema, H. G., Pfundt, R., Van Der Wilt, G. J., Brunner, H. G., Van Der Burg, S., Grutters, J. P., Veltman, J. A., & Willemsen, M. (2017). A clinical utility study of exome sequencing versus conventional genetic testing in pediatric neurology. Genetics in Medicine, 19(9), 1055–1063. https://doi.org/10.1038/gim.2017.1.
31. Smith, H. S., Swint, J. M., Lalani, S. R., Yamal, J., De Oliveira Otto, M. C., Castellanos, S., Taylor, A., Lee, B., & Russell, H. V. (2019). Clinical Application of Genome and Exome Sequencing as a Diagnostic Tool for Pediatric Patients: a Scoping Review of the Literature. Genetics in Medicine, 21(1), 3–16. https://doi.org/10.1038/s41436-018-0024-6.
32. Cakici, J. A., Dimmock, D., Caylor, S., Gaughran, M., Clarke, C., Triplett, C., Clark, M. M., Kingsmore, S. F., & Bloss, C. S. (2020). A Prospective Study of Parental Perceptions of Rapid Whole-Genome and-Exome Sequencing among Seriously Ill Infants. American Journal of Human Genetics, 107(5), 953–962. https://doi.org/10.1016/j.ajhg.2020.10.004.

SUPPLEMENTARY FIGURES AND TABLES



Supplementary Figure 1. Overview of all genetic tests
*Contains quantitative fluorescent polymerase chain reaction (QF-PCR) and fluorescence in situ hybridization (FISH). ES = exome sequencing.



Supplementary Figure 2. Enduser perspectives

Exploratory analysis to gain insight into the perspectives of parents of neonates receiving rapid ES as well as the involved clinicians (i.e. neonatologist). (A) The parental perspective was focused on their overall experience of the rapid ES procedure compared to routine testing. Overall experience was assessed by the visual analog scale (VAS), a 10-point scale ranging from 0 (extremely unsatisfied) to 10 (extremely satisfied) in which parents answered the following questions: 1) "To what extent are you satisfied about the rapid genetic diagnostic testing?"; and 2) "How satisfied would you be if genetic test results are available within 3-4 months (i.e. routine genetic diagnostic testing)?". In addition, to assess the perceived stress related to rapid ES, based on the differences in procedure such as the risk of incidental findings associated with rapid ES, parents indicated whether they agreed with the following two statements by using a 7-point scale ranging from completely agree to completely disagree: "It did not cause stress in order to decide whether rapid ES should be performed." (B) ; and 2) "If we had the opportunity to choose again for this diagnostic trajectory, we would choose again for rapid genetic diagnostic testing." (C). The involved clinicians' perspective was focused on the speed of rapid ES to have an impact on clinical decision-making (7-point scale, ranging from completely agree to completely disagree). They indicated to what extent they agreed with: "The turnaround time of rapid ES is short enough to impact clinical decision-making." (D). These exploratory questions and answer suggest that i) parents favoured the rapid ES over routine testing, ii) they experienced no extra stress related to testing and iii) that they would again choose rES if they had the opportunity. From the involved clinician's point-of-view, it seemed that the results from rapid ES were fast enough to impact clinical decision-making.

Supplementary Table 1
Supplementary Table 1 can be found online at
https://doi.org/10.1007/s00431-023-04909-1

Supplementary Table 2
Supplementary Table 2 can be found online at
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Supplementary Table 3
Supplementary Table 3 can be found online at
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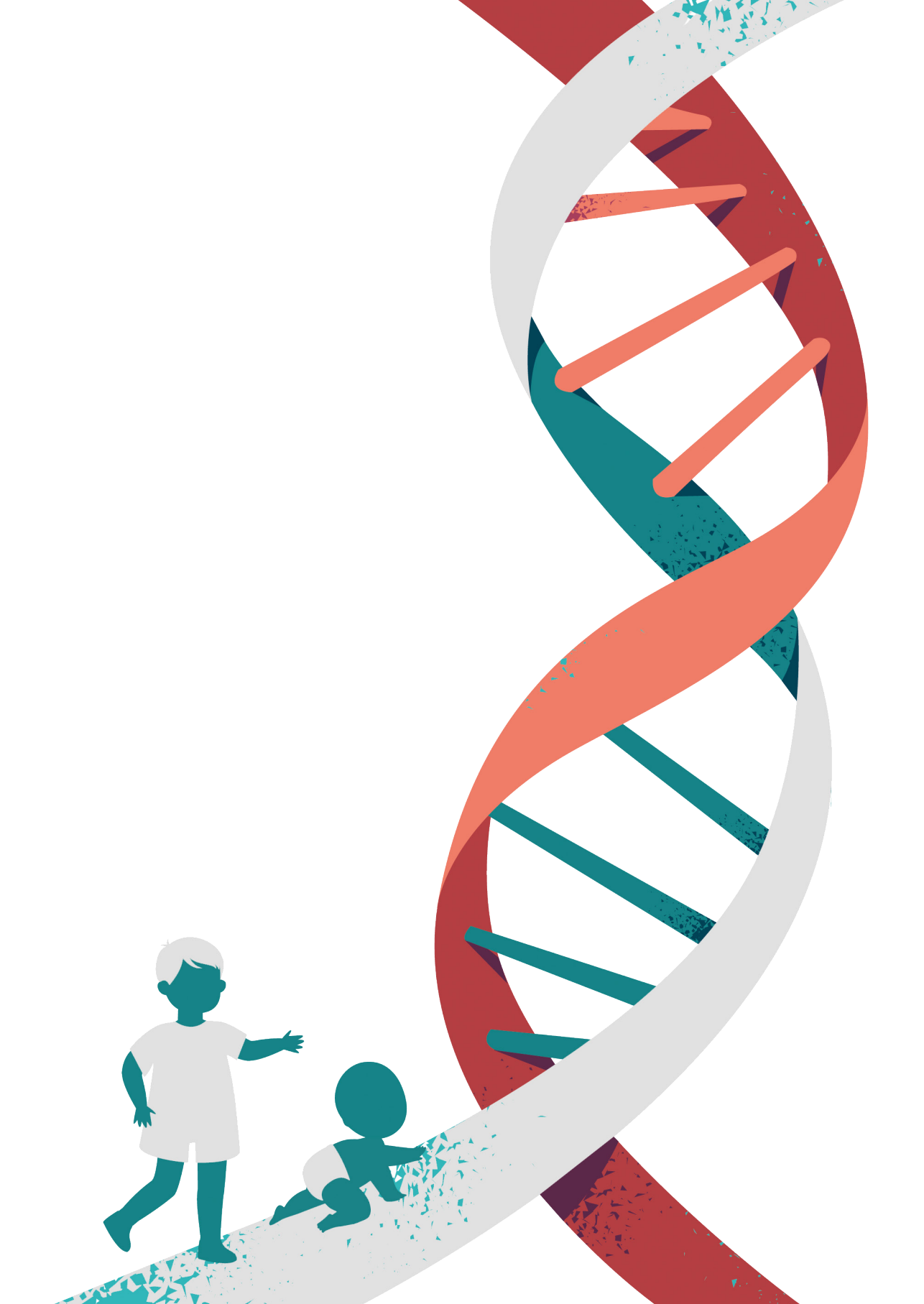


CHAPTER 6

General discussion

The overall aim of this thesis was to assess the economic value of implementing exome sequencing (ES) compared to current genetic diagnostic testing. Since clinical symptoms of rare diseases occur most often (i.e. approximately 75% of the cases) during childhood and since 80% of the rare diseases have a genetic origin [1-9], the impact of implementing ES within pediatric care was evaluated. This chapter will describe and discuss the main findings of this thesis, followed by a discussion of the methodological considerations and recommendations for future research and implications for practice.

6.1 MAIN FINDINGS

The implementation of next-generation sequencing (NGS) in pediatric care came with high expectations. Before implementing a new sequencing technology, these expectations make it essential to gain accurate insight in the added value, and require approaches to assess this added value (for example health or economic impact) appropriately in order to aid in decision-making. The first question we aimed to answer in this thesis was about choosing the optimal approach for economic evaluations of next generation sequencing in pediatric care. A review was performed (**chapter 2**), showing that currently used methods for economic evaluations lack uniformity and hamper reputable comparison between outcomes, thereby not efficiently aiding in decision-making. In order to facilitate and improve decision-making for implementing novel genetic technologies such as ES, it is of utmost importance to develop specific guidance of evaluations in pediatric care in addition to current health economic guidance.

In **chapter 3**, we described the improvement of the genetic diagnostic trajectory for critically ill neonates admitted to the neonatal intensive care unit (NICU) needed in order to provide a more inclusive, earlier and faster genetic diagnosis. There was a substantial proportion of patients, especially those patients with congenital anomalies (CA), who did not receive any genetic testing, without the presence of a possible explanation for this lack of testing. The limited uptake of genetic testing indicates the need for improved genetic diagnostic care in this setting. Especially patients with CA might benefit from the implementation of ES, since it was speculated that 29% of the patients with CA who did not receive genetic testing, probably remained undiagnosed due to a lack of genetic testing. Implementation of ES for all patients with CA at the NICU would overcome this problem and might end the diagnostic odyssey for (families of) these patients. Besides, by ending the diagnostic odyssey, personalized medicine can be enabled and/or improved, for example by starting the correct treatment or preventing unnecessary or risky therapies or change the goal of care, e.g. transition to comfort care.

This thesis shows that costs related to genetic diagnostic testing are only a fraction (2-5%) of the total healthcare costs for children admitted to the neonatal intensive care unit (NICU; **chapters 4 and 5).** The clinical impact, i.e. the impact on clinical outcome measurements, of implementing ES instead of conventional genetic testing, i.e. routine genetic diagnostic testing, can be significant. Both the number of patients who receive genetic testing and the diagnostic yield are likely to increase in case ES is implemented, without significantly increasing (overall) healthcare costs.

In the timeframe of performing the studies described in this thesis, the use of rapid ES in (neonatal) intensive care units has taken flight, even prior to the formal evaluation of the cost-effectiveness, as was highlighted in **chapter 5**. This overall suggests that the perceived opportunities of the ES in practice outweighed the challenges. This implies furthermore that, in addition to formal evaluations, early economic evaluations become even more important in this field. Evaluations in which room for improvement is calculated to outline possible added value before formal implementation and evaluations take place.

6.2 OPPORTUNITIES FOR ES

Previous research has outlined the potential impact of NGS in pediatric care on both costs and effects. Looking into current evidence, many opportunities have been described and are confirmed in this thesis. Most prevalent and essential opportunities are:

Ending the diagnostic odyssey

With the introduction of (rapid) ES as part of the genetic diagnostic trajectory, there might come an end to the diagnostic odyssey for the pediatric population, and in particular children admitted to the neonatal intensive care unit (NICU; **chapters 3 and 5**). As mentioned, this thesis (**chapter 5**) showed that implementation of rapid ES resulted in a larger number of patients who received a conclusive genetic diagnosis compared to routine genetic diagnostic testing (diagnostic yield of 20% vs. 10% respectively) with a shorter turnaround-time (15 days (95% CI 10–20) vs. 59 days (95% CI 23–98, $p<0.001$), respectively). This decrease in turnaround-time is in line with earlier research, which stated that implementation of rapid ES will shorten the diagnostic odyssey [10-12]. Regarding the diagnostic yield, this thesis showed a relatively lower diagnostic yield for rapid ES compared to similar studies in neonatal setting [13, 14]. This lower diagnostic yield was probably caused by the less strict inclusion criteria applied in **chapter 5**, since all neonates with a suspicion of a genetic disorder were included instead of a more specific study population. Besides, preselection was performed by pediatricians instead of clinical geneticists. Although there is a difference in results between previous studies and this thesis [13, 14], all studies have shown an increase in diagnostic yield

and decrease in turnaround-time, leading to an end of the diagnostic odyssey [10-14].

Decrease in healthcare costs at individual patient level

Results of **chapters 4** and **5** have shown that implementation of ES can be promising for critically ill neonates admitted to the NICU, without a significant increase in costs. The scenario analysis presented in **chapter 4** resulted in a small increase in overall healthcare costs, i.e. 5.3-5.5% increase in costs, in case all patients with congenital anomalies admitted to the NICU receive ES. **Chapter 5** showed net healthcare savings, i.e. a genetic cost reduction of 1.5%, when routine genetic diagnostic testing is replaced by rapid ES for patients admitted to the NICU.

Although the existing evidence regarding the cost-effectiveness of ES within pediatrics is relatively scarce, multiple studies have been performed, showing similar results compared to the results of this thesis [15]. These studies also showed that implementation of ES as first-tier test can be cost saving and/or might result in an increased number of conclusive diagnoses without a significant increase in costs [11, 12, 15, 16].

Enabling personalized medicine and prevention of unnecessary health care resource use

In this thesis, healthcare utilization and associated costs after a genetic diagnosis was established were not investigated. However, earlier research has shown that establishing a genetic diagnosis earlier in the diagnostic trajectory can positively affect clinical management of patients with rare diseases [17]. An earlier genetic diagnosis also enables personalized medicine, i.e. other extra or unnecessary invasive diagnostic tests can be prevented and future multidisciplinary care planning can be directed based on the genetic diagnosis [1, 18, 19].

By ending the diagnostic odyssey and enabling personalized medicine, healthcare resource use can be planned more efficiently [17]. For instance, an early diagnosis of a neonatally lethal disorder may lead to refraining from invasive open-heart surgery, whereas simultaneously, an early diagnosis of a disorder with later childhood presentation of comorbidities may lead to early intervention and prevention [17]. Such possible effects again outline the necessity of gaining early insight in possible cost savings as it is likely that such impact cannot be prospectively tested in formal randomized controlled trial studies and needs early health economic modelling to extrapolate and model current outcomes to future impact.

6.3 CHALLENGES RELATED TO THE IMPLEMENTATION OF ES

Despite the opportunities offered by the implementation of ES, the introduction of a new genomic technology such as ES is also associated with multiple challenges. Although ES has meanwhile been implemented in different settings including pediatric care, some important hurdles have been noted, and should be taken into account for future studies, cost evaluations, and decisions on widespread implementation.

Methodological challenges

One of the biggest lessons learned in this thesis was the lack of guidelines on how to perform an economic evaluation within the genetic pediatric population (or genetic testing in general) and how to create more uniformity regarding comparability of results (**chapter 2**). A more homogeneous methodology would increase comparability of results, and although there are specific guidelines on how to perform economic evaluations [20, 21], it seemed rather difficult to define the most optimal method in order to create more uniformity.

One of the insights obtained from previous studies is that quality-adjusted life years (QALY), a measure commonly used in health economic evaluations [22], seems to be inadequate: this measure may well work for the evaluation of adult interventions but is not directly applicable for children and neonates with a rare disease. Moreover, a diagnosis itself cannot be measured in QALYs, and often, for individuals with rare disease, and/or parents/caregivers perceived improvements in quality of life are much more subjective, and hard to capture in concise absolute numbers. More deliberate discussions should take place to ensure outcome measures are created which can be used by policymakers in the near future.

Moreover, as previously indicated, it is essential to develop high quality health economic models to extrapolate and model long term outcomes, as the time frame required to measure improvements is much longer than the current trials. One may think for instance of siblings or other relatives of the affected index individuals, who may benefit from the diagnosis as this allows for informed reproductive choices (e.g. information on the recurrence risk).

At the moment, it is thus still challenging to define the perfect alternative outcome measurement for genetic testing and to develop valid health economic models extrapolating short term outcomes to long term outcomes. Such models should become available early in the entire development and implementation process of new applications, and preferable added to other criteria for evaluating a genetic test, for

example, as defined by the Centers for Disease Control and Prevention ACCE framework (Acronym for: Analytical validity, Clinical validity, Clinical utility, and related Ethical/legal/social issues) [23]. These early economic evaluations can guide further decision-making before high quality efficiency outcomes are present and decrease the risk of making wrong decisions when evidence is limited. In addition to formal use of these evaluation it is essential to draft and develop cyclic health technology assessments in which evidence and decisions are updated over time.

Balance between costs of genetic testing, other healthcare utilization, and overall increase in volume of individuals using genetic services

As mentioned, implementing ES led to a non-significant change in genetic costs (**chapters 3** and **5**) as it replaces other genetic tests. It should however be noted that, although this is true at individual patient level, the new opportunities of genetic testing impact medical decision-making more broadly. The overall number of individuals making use of genetic testing has increased substantially (annual increase of 5-10%) and genetic testing has become more mainstream, i.e. increasingly offered within routine clinical practice and by non-genetic health professionals. In contrast, the overall budget a genetic center has available – based on negotiations with health care insurance companies via the Board of Directors from an academic hospital – is currently not equally compensated for the increase in volumes of testing (monetary increase is usually limited by inflation). Hence, to compensate for the increase in volume of testing, the costs of a new genetic test preferably need to decrease rather than being cost-neutral. Moreover, potential cost savings by intervening with medical treatments based on the genetic outcome, are often obtained in departments other than the genetic department itself (e.g. performing less cardiac screening is a cost saving for the department of Cardiology).

Genetic testing making headway, and being performed at an early moment in the care pathway for many more patients, thus to measure cost-effectiveness and develop decision analytic models early in the process as highlighted above not only calls for unified strategies, but also for a reevaluation of budgets, where costs are made, where cost savings end up, and how these are distributed.

Economic impact of potential increased health care utilization

Having a conclusive genetic diagnosis does not always involve manifestation of (all) clinical symptoms. Although a diagnosis can lead to an early start of treatment, there is also a risk of unnecessary healthcare utilization and unnecessary treatment, especially if a patient would have never developed the comorbidity being preventatively screened, or treated, for.

In this thesis, we did not analyze healthcare utilization after a diagnosis was established. To assess the impact of implementing a new genetic technology, it is important to be as precise as possible and include at least the complete diagnostic trajectory in a cost analysis (**chapter 2**). However, in order to be able to evaluate long-term economic impact, it also relevant to take into account the consequences following a conclusive diagnosis. When no consequences take place, and only consequence is knowing the diagnosis it should be questioned whether this is always the best and most cost-effective option. As mentioned, when a disease does not manifest itself (fully) and unnecessary treatment is started or a patient is referred to a specialist for follow-up, it can lead to an increase in healthcare costs. Especially in case a variance of unknown sequence (VUS) or incidental finding is detected (i.e. an unexpected finding indicating a pathogenic variant other than the indication for which the sequencing test was ordered) [24]. However, it remains rather challenging to study this impact, as previously outlined, and early health economic modelling could be an efficient solution to this issue. Both clinical and economic impact as outlined above, should be tested and evaluated in different scenarios in these models for instance.

Impact of implementing ES on parents

Another challenge that is related to the implementation of ES is the impact on parents and their experienced burden of the diagnostic trajectory. Both parents and patients may suffer from the diagnostic odyssey. Earlier research has shown that finding a conclusive diagnosis reduces emotional burden, validates the concerns of parents and allows access to established support networks for (parents of) patients without a diagnosis [25]. This can also positively affect daily functioning of both parents and patients.

In **chapter 5**, we tried to assess the experienced burden of parents of the patients caused by the genetic diagnostic trajectory and the impact of rapid ES instead of routine genetic testing on this burden. Compared to routine genetic testing, parents preferred rapid ES, rapid ES did not increase parental experienced stress and in general, parents evaluated rapid ES as having a positive impact on clinical decision-making. As these observations were rather exploratory in absence of validated questionnaires, making it challenging to conclude whether the impact of ES is significantly better, one might also argue that having diagnosis relieves stress. Previous studies confirm the positive attitudes of parents towards ES in the NICU [26]. The fact that these positive attitudes are more often obtained when using ES compared to previous standard of care may suggest that implementing ES has a reduced impact on the experienced burden of parents. This rational does, however, not take into account non-penetrant diseases, which may increase uncertainty, and/or the worries of parents may have on expression of the disease themselves, and/or the knowledge that they may have of transmitting the disease to their (next) child. Whereas these arguments are also of relevance for

diagnoses made from routine genetic testing, the number of parents dealing with such potential challenges does increase as a consequence of the increased diagnostic yield by using ES. This will result in a larger societal challenge.

Ethical considerations related to incidental findings and impact on health care utilization

Another impact aspect where ES differs from routine (targeted) genetic testing, is the chance of incidental findings, referring to a genetic finding that is of medical relevance, but is not the underlying cause of the condition the ES was performed for. Since their first discovery, guidelines were developed [27], which state that such findings should be reported in case future health can be influenced ("actionable"), for example, by starting (preventative) treatment. This is in line with the guideline developed by the Vereniging Klinische Genetica Nederland (VKGN) in the Netherlands [28], although international debates are still ongoing on whether one should actively screen for these variants or not [29]. In the Netherlands, depending on the diagnostic strategy to analyze ES data, the risk on identifying an incidental finding ranges between 0.04-1.03% [30]. Discussions on whether or not to disclose incidental findings are usually held in the context of the ethical considerations, balancing the potential harm of knowing versus the potential benefits of early intervention and/or life choices. In the context of this thesis, one may also consider emphasis on the health care utilization related to disclosure of incidental findings, especially as these genetic findings are usually related to disease segregating in (larger) families. This not only extends increased health care utilization beyond the index case, but also potentially leads to overdiagnosis and unnecessary treatments [31]. Hence, for an inclusive cost-evaluation of ES implementation, more detailed studies on the economic consequences of reporting incidental findings should be performed in the near future. Of note, we initially aimed to assess the impact of an incidental finding on healthcare utilization as part of this thesis, but unfortunately, the number of patients was too few and heterogeneous, and the follow-up time was too short to provide meaningful data for this thesis.

Generalizability of results across nations

Another important challenge regarding the implementation of ES is to combine study results across different countries. Apart from the methodological challenges highlighted in **chapter 2** and above, it should be realized that even if studies were comparable regarding effect measures and/or outcome measurements, the outcomes may not be directly translatable to local/national implementation strategies. This latter notion derives from different economic systems in each country, different reimbursement strategies, access to genetic services and general health care infrastructure. Hence, despite the strong encouragement to unify and homogenize economic evaluations of genetic testing, policy and decisions makers may still require dedicated national outcome

measures in a cost analysis for final decision-making.

6.4 RECOMMENDATIONS FOR FUTURE RESEARCH AND PRACTICE

Multiple recommendations for future research and practice can be defined based on the opportunities and challenges presented in this thesis.

Focus on long term health care utilization

The implementation of (rapid) ES for critically ill neonates admitted to the NICU has been effected during the course of the studies described in this thesis, mostly based on direct short term clinical benefits (e.g. increased diagnostic yield, and impact on clinical decision-making) without obvious incurred higher health care costs. The trend of genetics playing its part in almost all medical disciplines, will lead to an increase of the number of individuals receiving testing. Longitudinal studies (retrospective or prospective) on health care utilization will provide much needed insights into the impact of long-term clinical and economic consequences of new genetic technologies.

These longitudinal studies should preferably involve novel outcome measures (e.g. other than QALY) that are specific and normative to evaluate all relevant socio-economic aspects of implementing the innovative genetic technology. In this context one might also investigate differences observed for those individuals with a diagnosis and without a diagnosis, and those differences related to primary diagnosis, and those related to incidental findings. More (early) health economic models should therefore come available which are transparent, regularly updated and easily transferable to other countries. Collaboration should therefore take place within countries but also with health economic experts in different countries.

Evaluation of novel technologies in the context of the fast-evolving field of medical genetics

The possibilities ES has offered the field of medical genetics are enormous. Yet, the technological revolution is still ongoing, and includes the analysis of the genome over the exome using various platforms (short reads versus long read sequencing), different biological samples (transcriptome and metabolome), but also alternative non-sequencing-based strategies (optical genome mapping). Each of these strategies and applications will have a sweet spot for its use in a genetic diagnostic laboratory to diagnose rare disease. It is as such of essence that the main lessons learned from the studies described in this thesis are leveraged onto novel studies to assess where these sweet spots lie to address the question: which patient benefits most, from which diagnostic strategy, in a viable socio-economic health care system. Whereas ES has been

implemented based on the aforementioned direct clinical benefits, it might be expected that the direct added value of each novel technique might be smaller. In addition, currently, the direct consumable costs and costs for infrastructure exceed those of ES. Hence, the timing is now to design studies to assess whether, and if so, under which circumstances, these benefits should outweigh the possible extra costs. Development and use of early economic evaluations is therefore essential to gain early insight in possible added value and pinpoint clinical studies on these areas which are likely to have the highest impact.

Paying attention to indirect consequences and costs associated with implementing novel technologies

In addition to costs directly visible and quantifiable from implementation of new genetic diagnostic strategies, such as for instance new equipment and analytical software, policymakers should also pay attention to indirect costs and consequences associated with implementation. For instance, with the implementation of ES, time and money have been spent on training of personnel and educating other healthcare professionals [32, 33]. Also, communication plans and strategies are required to inform all stakeholders for the need for innovation. In the case of ES, the direct added clinical benefit, early initiation of treatment etc., was generally appreciated and clinicians were willing to accept ES [10]. However, without a clear explanation of the possible advantages or disadvantages, it can be challenging to motivate these clinicians to implement a genetic innovation since they are used to routine genetic care.

Discussion about the added benefit holds for other stakeholders such as (parents of) patients and their families as well. As mentioned earlier, ES can be associated with possible negative impacts, for example incidental findings [24]. Parents and patients should be aware of both the possible clinical benefit and potential risks and the possibility of incidental findings [34] and should also maintain realistic expectations. Implementation of ES does not guarantee a conclusive diagnosis and there is always a possibility of being confronted with an insecure outcome or an outcome which does not have treatment options yet. Sufficient guidance is needed in order to let (parents of a) patient decide whether ES should be performed.

Additional training, professional and public education and extra guidance will lead are related to a (temporary) increase of costs. However, within the Netherlands, genetics is more obviously integrated into medical practice, and healthcare professionals are more and more familiar with genetic testing. Although this reduces the extra costs needed to train the (non-) geneticists, overall costs related to training and education are currently unknown.

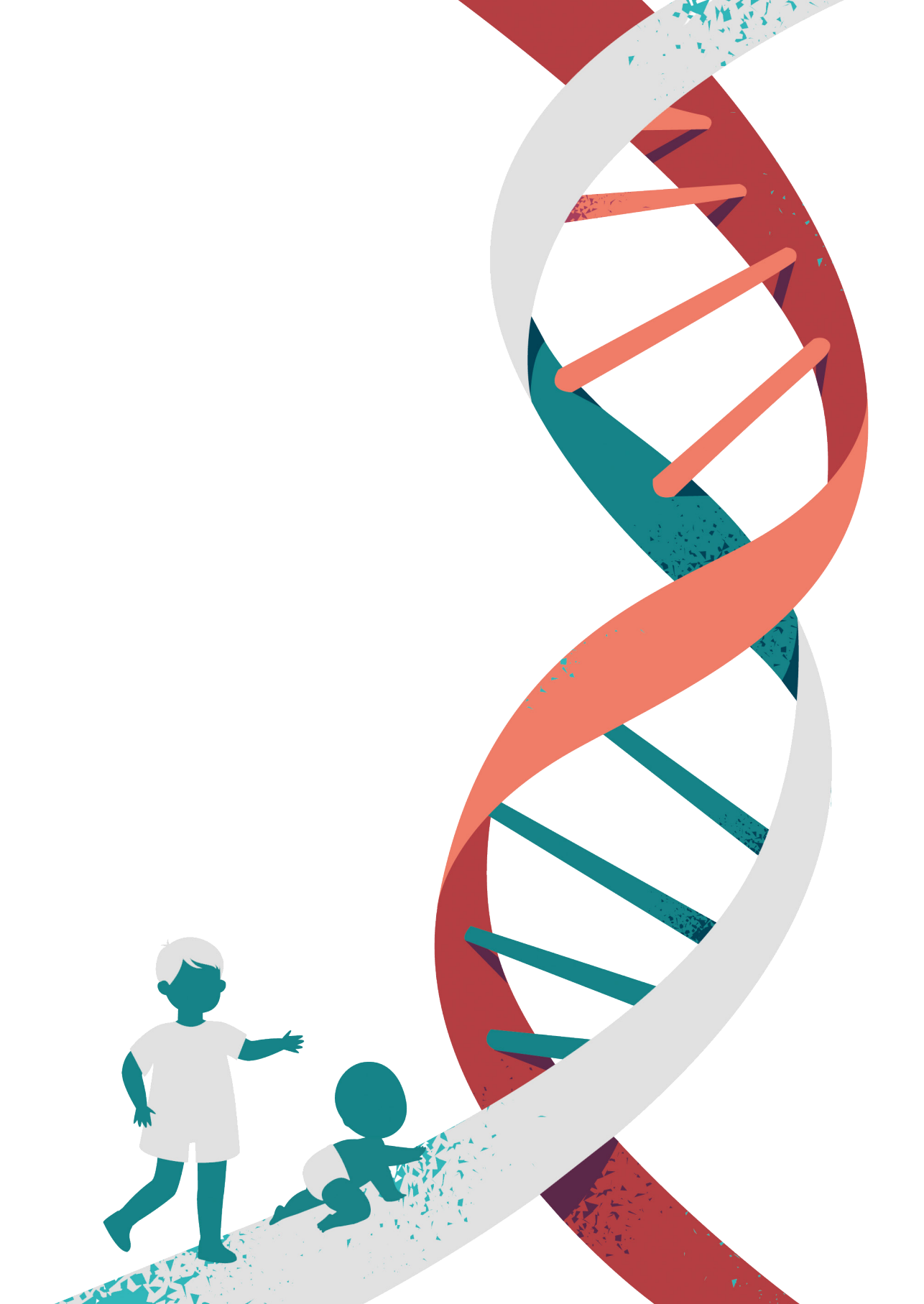
6.5 CONCLUDING REMARKS

This thesis has shown that exome sequencing has potential to become part of routine genetic testing for critically ill neonates admitted to the neonatal intensive care unit and as a first-tier diagnostic test. Implementation of exome sequencing does not significantly affect costs, especially when used for all patients with congenital anomalies. However, while performing the cost-effectiveness studies as presented, implementation of exome sequencing took place, without waiting for the outcomes of these studies. With the technological revolution in the field of medical genetics still ongoing, future cost-effectiveness analyses should therefore be performed earlier in the process towards implementation. Consequently, decisions on implementation need to be made based on these early health economic evaluations, preferably using valid and transparent decision analytic models outlining future costs and effects. Thereafter, these models should be updated when new real-world data becomes available and frameworks should be developed in order to enhance further implementation and even de-implementation of innovations within patient pathways that are ultimately deemed not cost-effective. This involves adding a layer of uncertainty (uncertainty related to data maturity) to diagnostic decision-making in the future. This uncertainty can be overcome using long-term data from other studies and high-quality data from current practice. Early understanding of potential added value is essential in this case to further steer and guide the future of innovative diagnostic modalities.

REFERENCES

1. Borghesi, A., Mencarelli, M. A., Memo, L., Ferrero, G. B., Bartuli, A., Genuardi, M., Stronati, M., Villani, A., Renieri, A., & Corsello, G. (2017). Intersociety policy statement on the use of whole-exome sequencing in the critically ill newborn infant. Italian Journal of Pediatrics, 43(1). https://doi.org/10.1186/s13052-017-0418-0.
2. Costa, S., Rodrigues, M., Centeno, M. J., Martins, A., Vilan, A., Brandão, O., & Guimarães, H. (2010). Diagnosis and cause of death in a neonatal intensive care unit - How important is autopsy? Journal of Maternal-fetal & Neonatal Medicine, 24(5), 760-763. https://doi.org/10.3109/14767058.2010.520047.
3. Simpson, C., Ye, X. Y., Hellmann, J., & Tomlinson, C. (2010). Trends in Cause-Specific mortality at a Canadian outborn NICU. Pediatrics, 126(6), e1538–e1544. https://doi.org/10.1542/peds.2010-1167.
4. Tyson, J. E., & Kennedy, K. A. (2002). Variations in mortality rates among Canadian neonatal intensive care units: interpretation and implications. PubMed, 166(2), 191–192. https://pubmed.ncbi.nlm.nih.gov/11826942.
5. Weiner, J., Sharma, J., Lantos, J. D., & Kilbride, H. W. (2011). How infants die in the Neonatal Intensive Care Unit. Archives of pediatrics & adolescent medicine, 165(7), 630. https://doi.org/10.1001/archpediatrics.2011.102
6. Wen, S. W., Liu, S., Joseph, K. S., Rouleau, J., & Allen, A. C. (2000). Patterns of infant mortality caused by major congenital anomalies. Birth Defects Research, 61(5), 342–346. https://doi.org/10.1002/(sici)1096-9926(200005)61:5.
7. Wojcik, M. H., Schwartz, T. S., Yamin, I., Edward, H. L., Genetti, C. A., Towne, M. C., & Agrawal, P. B. (2018). Genetic disorders and mortality in infancy and early childhood: delayed diagnoses and missed opportunities. Genetics in Medicine, 20(11), 1396–1404. https://doi.org/10.1038/gim.2018.17.
8. European Commission. Available from: https://ec.europa.eu/info/research-and-innovation/research-area/health-research-and-innovation/rare-diseases_en.
9. Boycott, K. M., Vanstone, M. R., Bulman, D. E., & MacKenzie, A. (2013). Rare-disease genetics in the era of next-generation Sequencing: Discovery to translation. Nature Reviews Genetics, 14(10), 681–691. https://doi.org/10.1038/nrg3555.
10. D'Gama, A. M., Del Rosario, M. C., Bresnahan, M., Yu, T. W., Wojcik, M. H., & Agrawal, P. B. (2022). Integrating rapid exome sequencing into NICU clinical care after a pilot research study. npj Genomic Medicine, 7(1). https://doi.org/10.1038/s41525-022-00326-9.
11. Tsang, M. H., Chiu, A. T. G., Kwong, B. M. H., Liang, R., Yu, M. H., Yeung, K. S., Ho, W. H. L., Mak, C. C. Y., Leung, G. K. C., Pei, S. L. C., Fung, J. L., Wong, V., Muntoni, F., Chung, B. H., & Chan, S. H. (2020). Diagnostic value of whole-exome sequencing in Chinese pediatric-onset neuromuscular patients. Molecular Genetics & Genomic Medicine, 8(5). https://doi.org/10.1002/mgg3.1205.
12. Vissers, L. E., Van Nimwegen, K., Schieving, J., Kamsteeg, E. J., Kleefstra, T., Yntema, H. G., Pfundt, R., Van Der Wilt, G. J., Brunner, H. G., Van Der Burg, S., Grutters, J. P., Veltman, J. A., & Willemsen, M. (2017). A clinical utility study of exome sequencing versus conventional genetic testing in Pediatric neurology. Genetics in Medicine, 19(9), 1055–1063. https://doi.org/10.1038/gim.2017.1.
13. Kapil, S., Fishler, K. P., Euteneuer, J. C., & Brunelli, L. (2018). Many newborns in level IV NICUs are eligible for rapid DNA sequencing. American Journal of Medical Genetics - Part A, 179(2), 280–284. https://doi.org/10.1002/ajmg.a.61011.
14. Stark, Z., Tan, T. Y., Chong, B., Brett, G. R., Yap, P., Walsh, M., Yeung, A., Peters, H., Mordaunt, D., Cowie, S., Amor, D. J., Savarirayan, R., McGillivray, G., Downie, L., Ekert, P. G., Theda, C., James, P., Yaplito-Lee, J., Ryan, M. M., . . . White, S. (2016). A prospective evaluation of whole-exome sequencing as a first-tier molecular test in infants with suspected monogenic disorders. Genetics in Medicine, 18(11), 1090–1096. https://doi.org/10.1038/gim.2016.1.
15. Schwarze, K., Buchanan, J., Taylor, J. C., & Wordsworth, S. (2018). Are whole-exome and whole-genome sequencing approaches cost-effective? A Systematic review of the literature. Genetics in Medicine, 20(10), 1122–1130. https://doi.org/10.1038/gim.2017.247.
16. Deden, C., Neveling, K., Zafeiropopoulou, D., Gilissen, C., Pfundt, R., Rinne, T., De Leeuw, N., Faas, B. H. W., Gardeitchik, T., Sallevelt, S. C. E. H., Paulussen, A. D. C., Stevens, S. J., Sikkel, E., Elting, M. W., Van Maarle, M. C., Diderich, K. E. M., Corsten-Janssen, N., Lichtenbelt, K. D., Lachmeijer, G., . . . Van Zelst-Stams, W. A. G. (2020). Rapid whole exome sequencing in pregnancies to identify the underlying genetic cause in fetuses with congenital anomalies detected by ultrasound imaging. Prenatal Diagnosis, 40(8),

972–983. https://doi.org/10.1002/pd.5717.
17. Williamson, S., Rasanayagam, C., Glover, K., Baptista, J., Naik, S., Satodia, P., & Gowda, H. (2021). Rapid exome sequencing: revolutionises the management of acutely unwell neonates. European Journal of Pediatrics, 180(12), 3587–3591. https://doi.org/10.1007/s00431-021-04115-x.
18. Joshi, C., Kolbe, D. L., Mansilla, M. A., Mason, S. O., Smith, R. J., & Campbell, C. A. (2016b). Reducing the cost of the diagnostic odyssey in early onset epileptic encephalopathies. BioMed Research International, 2016, 1–8. https://doi.org/10.1155/2016/6421039.
19. Petrikin, J. E., Willig, L. K., Smith, L. D., & Kingsmore, S. F. (2015). Rapid whole genome sequencing and precision neonatology. Seminars in Perinatology, 39(8), 623–631. https://doi.org/10.1053/j.semperi.2015.09.009.
20. Bick, D., & Dimmock, D. (2011). Whole exome and whole genome sequencing. Current Opinion in Pediatrics, 23(6), 594–600. https://doi.org/10.1097/mop.0b013e32834b20ec.
21. Buchanan, J., Wordsworth, S., & Schuh, A. (2013). Issues surrounding the health economic evaluation of genomic technologies. Pharmacogenomics, 14(15), 1833–1847. https://doi.org/10.2217/pgs.13.183.
22. Drummond, M.F., et al., Methods for economic evaluation of health care programmes. 4 ed. 2015, Oxford: Oxford University Press.
23. Haddow, J.E., Palomaki, G.E. , & Khoury, M. (2004). Human genome epidemiology: A scientific foundation for using genetic information to improve health and prevent disease, in ACCE: a model process for evaluating data on emerging genetic tests.
24. G Green, R. C., Berg, J. S., Grody, W. W., Kalia, S. S., Korf, B. R., Martin, C. L., McGuire, A. L., Nussbaum, R. L., O'Daniel, J., Ormond, K. E., Rehm, H. L., Watson, M. S., Williams, M. S., & Biesecker, L. G. (2013). ACMG recommendations for reporting of incidental findings in clinical exome and genome sequencing. Genetics in Medicine, 15(7), 565–574. https://doi.org/10.1038/gim.2013.73.
25. Carmichael, N., Tsipis, J., Windmueller, G., Mandel, L., & Estrella, E. (2014). "Is it going to hurt?": The impact of the diagnostic odyssey on children and their families. Journal of Genetic Counseling, 24(2), 325–335. https://doi.org/10.1007/s10897-014-9773-9.
26. Berrios, C., Koertje, C., Noel-Macdonnell, J. R., Soden, S. E., & Lantos, J. D. (2020). Parents of newborns in the NICU enrolled in genome sequencing research: hopeful, but not naïve. Genetics in Medicine, 22(2), 416-422. https://doi.org/10.1038/s41436-019-0644-5.
27. Kalia, S. S., Adelman, K., Bale, S. J., Chung, W. K., Eng, C. M., Evans, J. P., Herman, G. E., Hufnagel, S. B., Klein, T. E., Korf, B. R., McKelvey, K. D., Ormond, K. E., Richards, C. S., Vlangos, C. N., Watson, M. S., Martin, C. L., & Miller, D. T. (2017). Recommendations for Reporting of Secondary Findings in Clinical exome and Genome Sequencing, 2016 Update (ACMG SF v2.0): A Policy statement of the American College of Medical Genetics and Genomics. Genetics in Medicine, 19(2), 249–255. https://doi.org/10.1038/gim.2016.190.
28. Vereniging Klinische Genetica Nederland (VKGN, 2020). Consensus-based leidraad voor het melden van nevenbevindingen in de klinisch genetische diagnostiek. Available from: https://www.vkgn.org/nieuws/landelijk-beleid-voor-het-melden-van-nevenbevindingen-in-de-klinisch-genetische-diagnostiek/.
29. Haer-Wigman, L., Van Der Schoot, V., Feenstra, I., Silfhout, A. T. V., Gilissen, C., Brunner, H. G., Vissers, L. E. L. M., & Yntema, H. G. (2018). 1 in 38 individuals at risk of a dominant medically actionable disease. European Journal of Human Genetics, 27(2), 325–330. https://doi.org/10.1038/s41431-018-0284-2.
30. Van Der Schoot, V., Haer-Wigman, L., Feenstra, I., Tammer, F., Oerlemans, A., Van Koolwijk, M. P. A., Van Agt, F., Arens, Y., Brunner, H. G., Vissers, L. E., & Yntema, H. G. (2021). Lessons learned from unsolicited findings in clinical exome sequencing of 16,482 individuals. European Journal of Human Genetics, 30(2), 170–177. https://doi.org/10.1038/s41431-021-00964-0.
31. Welch, H. G., & Black, W. C. (2010). Overdiagnosis in cancer. Journal of the National Cancer Institute, 102(9), 605–613. https://doi.org/10.1093/jnci/djq099.
32. Mellis, R., Tapon, D., Shannon, N., Dempsey, E., Pandya, P., Chitty, L. S., & Hill, M. (2022). Implementing a rapid fetal exome sequencing service: What do parents and health professionals think? Prenatal Diagnosis, 42(6), 783–795. https://doi.org/10.1002/pd.6140.
33. Félix, T. M., De Souza, C. F. M., Oliveira, J. B., Rico-Restrepo, M., Zanoteli, E., Zatz, M., & Giugliani, R. (2023). Challenges and recommendations to increasing the use of exome sequencing and whole genome sequencing for diagnosing rare diseases in Brazil: An Expert perspective. International Journal for Equity in Health, 22(1). https://doi.org/10.1186/s12939-022-01809-y.
34. Levenseller, B., Soucier, D., Miller, V. A., Harris, D., Conway, L., & Bernhardt, B. A. (2013). Stakeholders' opinions on the implementation of Pediatric whole exome sequencing: Implications for informed consent. Journal of Genetic Counseling, 23(4), 552–565. https://doi.org/10.1007/s10897-013-9626-y.



Appendices

LIST OF ABBREVIATIONS

aCHG	Microarray-based comparative genomic hybridization
ACMG	American College of Medical Genetics
CA	Congenital anomaly
CEA	Cost-effectiveness analysis
CMA	Chromosomal microarray
CNV	Copy number variation
EMR	Electronic medical record
EQ-5D	EuroQol-5D
ES	Exome sequencing
FISH	Fluorescence in situ hybridization
GEECS	Global Economics and Evaluation of Clinical Genomics Sequencing Working Group
GS	Genome sequencing
HPO	Human phenotype ontology
ICER	Incremental cost-effectiveness ratio
MCA	Multiple congenital anomalies
MLPA	Multiplex ligation-dependent probe amplification
NDD	Neurodevelopmental disorders
NGS	Next-generation sequencing
NICE	National Institute for Health and Clinical Excellence
NICU	Neonatal intensive care unit
NIPT	Noninvasive prenatal testing
NZA	Nederlandse Zorgautoriteit
PCR	Polymerase chain reaction
PICU	Pediatric intensive care unit
QALY	Quality-adjusted life year
QF-PCR	Quantitative fluorescent polymerase chain reaction
SNP	Single nucleotide polymorphism
SNV	Single nucleotide variant
STR	Short tandem repeat
TAT	Turnaround time
TTD	Time-to-diagnosis
UPD	Uniparental disomy
VAS	Visual Analogue Scale
VKGN	Vereniging Klinische Genetica Nederland
VUS	Variance of unknown significance
ZIN	Zorginstituut Nederland

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SUMMARY

In the last decade, the landscape of genetic testing has changed enormously. Genetic sequencing technologies are increasingly used for the diagnosis of rare genetic disorders. Exome sequencing (ES) is emerging as powerful diagnostic tool in identifying the genetic etiology of a rare disease, especially within pediatrics. There is however still an unmet need to better understand the economic impact of ES implementation in routine care, also including other possible benefits of ES, such as improvements in clinical decision-making, and reducing the burden to families. A cohort of patients in which these economic outcomes and clinical benefits might be best captured are neonates admitted to neonatal intensive care units (NICUs). For these neonates, it is expected that genetic test results may impact clinical decision-making and enable personalized medicine. Yet, genetic testing using ES has not reached widespread implementation in this setting. This is mainly due to the need of NICUs to have a fast ES result, requiring infrastructure on Dutch genetic laboratories that have only been set up recently. Since there are also still uncertainties regarding effect/outcome measures to be taken into account for economic evaluation when implementing novel genetic tests, the overall aim of this thesis was to assess the economic impact of implementing ES in routine care for children admitted to the NICU.

In **chapter 2**, a systematic literature review was conducted (14 February 2021), aimed at identifying all published economic evaluations of sequencing technologies in pediatric diagnostics, i.e. ES and/or genome sequencing (GS). Information regarding methodological approach, costs, effects, and time horizon was abstracted from these publications. Overall, twenty-eight economic evaluations of ES/GS within pediatrics were identified. Costs included were mainly restricted to direct in-hospital healthcare costs and varied widely in inclusion of sort of costs and time horizon. Nineteen studies included diagnostic yield and eight studies included cost-effectiveness as outcome measures. Studies varied greatly in terms of included sort of costs data, effects, and time horizon. Due to the large differences in inclusion of costs and effect parameters between studies, the validity of outcomes can be questioned. This hinders valid comparison and widespread generalization of conclusions. In addition to current health economic guidance, specific guidance for evaluations in pediatric care is therefore necessary to improve the validity of outcomes and furthermore facilitate comparable decision-making for implementing novel NGS-based diagnostic modalities in pediatric genetics and beyond.

Since it has been proven that neonates with genetic disorders or congenital anomalies contribute considerably to morbidity and mortality in NICUs, the exact prevalence of genetic disorders in an academic level IV NICU was assessed in **chapter 3**. Clinical

and genetic data was retrospectively collected data from 1,444 infants admitted to an academic hospital in the Netherlands between October 2013 to October 2015. Follow up of these patients continued until infants reached at least two years of age. Results have shown that for 13% (194/1,444) of all patients genetic tests were performed, resulting in a diagnostic yield of 37% (72/194). In 53% (38/72), the diagnosis was made post-neonatally (median age = 209 days) using assays including ES. 32% (461/1,444) of the patients suffered from a congenital anomaly. 63% (291/461) of these patients never received genetic testing, despite being clinically similar those who did. Extrapolation of the diagnostic yield suggests that up to 6% (83/1,444) of our total cohort may have remained genetically undiagnosed, indicating that 29% (83/291) of not-tested patients with a congenital anomaly could have likely remained undiagnosed due to a lack of genetic testing. The data shown in chapter 3 indicates the need to improve genetic care in the NICU for more inclusive, earlier, and faster genetic diagnosis to enable tailored management.

The objective of **chapter 4** was to provide an overview of the average healthcare costs for patients admitted to the NICU and to assess the possible impact of implementing ES on these total healthcare costs. Post-natal healthcare data of all patients admitted to the level IV NICU at the Radboudumc (October 2013–October 2015) was collected and unit costs were linked to these healthcare consumptions. A distinction between patients was made based on performance of genetic tests and the presence of (multiple) congenital anomalies. Overall, on average €26,627 was spent per patient. Genetic costs accounted for 2.3% of all costs. Average healthcare costs were higher for patients with multiple congenital anomalies (€53,686 per patient) compared to patients with an isolated congenital anomaly (€27,350 per patient) and patients without congenital anomalies (€15,210 per patient). We next modelled four scenarios based on clinical preselection. First, when performing trio-ES for all patients instead of routine genetic testing, overall healthcare costs will increase with 22.2%. Second, performing trio-ES only for patients with multiple congenital anomalies will not result in any cost changes, but this would leave patients with an isolated congenital anomaly untested. We therefore next modelled a scenario performing trio-ES for all patients with congenital anomalies, increasing the average per patient healthcare costs by 5.3%. This will rise to a maximum of 5.5% when also modelling for an extra genetic test for clinically selected patients to establish genetic diagnoses that are undetectable by ES. Overall, genetic diagnostic testing accounted for a small fraction of total costs. Implementation of trio-ES as first-tier test for all patients with congenital anomalies will lead to a limited increase in overall healthcare budget, but will facilitate personalized treatments options guided by the diagnoses made.

Although clinical decision-making can be positively affected by the introduction of rapid ES for critically ill neonates admitted to the NICU, prospective evidence to quantify the

impact of rapid ES over routine genetic testing are scarce. In **chapter 5**, a prospective clinical utility study was performed, comparing rapid ES to conventional genetic testing. Five Dutch NICUs were involved and 60 neonates with a suspected genetic disorder were included in the study. For each participant, both rapid ES and routine genetic testing were performed in parallel. Both diagnostic yield and time-to-diagnosis were monitored. Healthcare resource use was collected to assess the economic impact of rapid ES. Questionnaires and Visual Analogue Scale (VAS) scores were obtained from parents and involved clinicians to gain insight into their perspectives of rapid ES. Compared to routine genetic testing, rapid ES detected significantly more conclusive genetic diagnoses (20% and 10% respectively, $P<0.05$), in a significantly shorter time-to-diagnosis (59 days (95% CI 22-99) vs. 15 days (95% CI 10-20) respectively). Regarding the costs, rapid ES reduced genetic diagnostic costs by 1.5% (€85 per neonate). Compared to routine genetic testing, parents showed preference towards rapid ES, and involved clinicians reported perceived clinical benefits for rapid ES. Together with the positive user experience, these observations warrant the widespread implementation of rapid ES as first-tier genetic test in critically ill neonates with disorders of suspected genetic origin.

Concluding remarks

Overall, this thesis has shown that exome sequencing has great potential (both clinical and economic) to become part of routine genetic testing for critically ill neonates admitted to the neonatal intensive care unit. Especially if exome sequencing is implemented as first-tier diagnostic test for all patients with congenital anomalies, it will not lead to a significant increase in healthcare costs. However, the implementation of exome sequencing did not await the outcomes of these studies. With the technological revolution in the field of medical genetics still ongoing, future cost-effectiveness analyses should be performed earlier in the process towards implementation. Based on these early health economic evaluations, decisions regarding implementation need to be made, preferably using valid and transparent decision analytic models outlining future costs and effects. Thereafter, these models should be updated as new real-world data becomes available, and frameworks should be developed in order to enhance further implementation and even de-implementation of patient pathways that are ultimately deemed not cost-effective. This includes adding a layer of uncertainty (uncertainty about data maturity) to future decision-making. The use of long-term data from other studies and high-quality data from current practice can remove the uncertainty and early insight in the potential added value is in this case essential to further steer and guide the future of innovative diagnostic modalities.

NEDERLANDSE SAMENVATTING

In het afgelopen decennium is het landschap van genetisch testen enorm veranderd. Genoombrede DNA-sequencing technologieën worden steeds vaker gebruikt voor het stellen van de diagnose van zeldzame genetische aandoeningen. Exoomsequencing (ES) is één van de methoden en is in opkomst als krachtig diagnostisch hulpmiddel bij het identificeren van de genetische etiologie van een zeldzame ziekte, vooral binnen de kindergeneeskunde. Er is behoefte om de economische impact van ES-implementatie in de routinezorg beter te begrijpen, ook met betrekking tot andere voordelen van ES, zoals verbeteringen in de klinische besluitvorming en het verminderen van de last van onzekerheid voor gezinnen. Een groep van patiënten waarin deze economische uitkomsten en klinische voordelen het beste tot hun recht komen, zijn pasgeborenen die zijn opgenomen op neonatale intensive care units (NICU's). Voor deze pasgeborenen wordt verwacht dat genetische testresultaten van invloed kunnen zijn op de klinische besluitvorming en gepersonaliseerde geneeskunde mogelijk maken. Toch hebben genetische testen gebaseerd op ES in deze setting nog geen algehele implementatie bereikt. Hiervoor is een infrastructuur vereist die in staat is om een snel ES-resultaat te krijgen. De Nederlandse genetische laboratoria hebben deze in de laatste jaren opgezet. Aangezien er voor de economische evaluatie bij het implementeren van nieuwe genetische testen onzekerheden zijn met betrekking tot effect-/uitkomstmaten waarmee rekening moet worden gehouden, was het algemene doel van dit proefschrift om de economische impact te beoordelen van het implementeren van ES in de routinematige zorg voor kinderen die zijn opgenomen op de NICU.

In **hoofdstuk 2** is een systematische literatuur review uitgevoerd (14 februari 2021), gericht op de identificatie van alle gepubliceerde economische evaluaties met betrekking tot sequencing technologieën binnen de pediatrische diagnostiek, in dit geval ES en genoomsequencing (GS). Van alle geïncludeerde publicaties is informatie verzameld met betrekking tot de methodologische aanpak, kosten, effectmaten en tijdsperiode waarin patiënten gevolgd zijn. In totaal zijn er 28 economische evaluaties van ES en/of GS binnen de pediatrische populatie geïdentificeerd. De kosten die in deze studies meegenomen werden, waren met name beperkt tot gezondheidskosten die binnen een ziekenhuis gemaakt worden. Een heldere omschrijving van de specifieke soort kosten ontbrak vaak en er was veel variatie in de tijdsperiode waarover kosten verzameld zijn. 19 studies gebruikten de diagnostische opbrengst als uitkomstmaat, terwijl 8 studies de kosteneffectiviteit als uitkomst omschreven. De omvang van de onderzochte variabelen (i.e. soort kostendata, uitkomstmaten en tijdsperiode waarover kosten zijn verzameld) was erg uiteenlopend. Vanwege deze variatie kan de validiteit van de uitkomsten in twijfel getrokken worden. Dit beperkt de mogelijkheden om onderzoeksresultaten te vergelijken en zorgt voor een afname van de generaliseerbaarheid van conclusies. Als aanvulling

op bestaande gezondheidseconomische richtlijnen is er behoefte aan specifieke richtlijnen voor evaluaties die worden uitgevoerd binnen de kindergeneeskunde. Dit kan een positieve impact hebben op de validiteit van uitkomstmaten en kan klinische besluitvorming bijvoorbeeld omtrent implementatie van nieuwe (sequencing) technologieën binnen de pediatrische genetica faciliteren.

Pasgeborenen met genetische aandoeningen of aangeboren afwijkingen dragen aanzienlijk bij aan ziekte en sterfte op de NICU. Daarom is de prevalentie van genetische aandoeningen binnen een academische level IV NICU onderzocht in **hoofdstuk 3**. Klinische en genetische data van 1.444 kinderen, opgenomen in een academisch ziekenhuis in Nederland tussen oktober 2013 en oktober 2015, zijn retrospectief verzameld. Gegevens werden verzameld tot de patiënten een minimale leeftijd van twee jaar hadden bereikt. De resultaten hebben laten zien dat genetisch onderzoek is verricht voor 13% (194/1.444) van alle patiënten. Dit heeft voor 37% (72/194) van de patiënten tot een diagnose geleid. In 53% (38/72) van de diagnosen is de diagnose post-neonataal gesteld (mediane leeftijd = 209 dagen). Hierbij zijn verschillende genetische testen ingezet, waaronder ES. Van alle patiënten leed 32% (461/1.444) aan een aangeboren afwijking. Voor 63% (291/461) van deze patiënten is er nooit genetische diagnostiek uitgevoerd, ondanks dat deze patiënten klinisch gezien gelijk waren aan de patiënten waarvoor wel genetische diagnostiek werd ingezet. Op basis van extrapolatie van de verwachtte diagnostische opbrengst kan gezegd worden dat tot 6% (83/1.444) van het totale cohort geen genetische diagnose heeft ontvangen. Dit komt overeen met 29% (83/291) van de patiënten met een aangeboren afwijking waarvoor geen genetische diagnostiek is ingezet. Deze patiënten hebben uiteindelijk geen definitieve diagnose gekregen wegens het niet inzetten van de genetische test. De resultaten van hoofdstuk 3 geven aan dat genetische zorg op de NICU verbeterd moet en kan worden. Hierdoor kan eerder en in een kortere tijd een genetische diagnose gesteld worden zodat zorg op maat gegeven kan worden.

Het doel van **hoofdstuk 4** was het in kaart brengen van de gemiddelde kosten van de gezondheidszorg voor pasgeborenen die opgenomen zijn op de NICU en het inzichtelijk maken van de mogelijke impact van de implementatie van ES op de totale kosten. Postnatale gezondheidszorgdata zijn verzameld van alle patiënten die waren opgenomen op een level IV NICU in het Radboudumc (oktober 2013-oktober 2015). Gezondheidszorggebruik werd gekoppeld aan de kostprijs. Er werden verschillende patiëntgroepen gemaakt op basis van het wel/niet uitvoeren van genetische diagnostiek voor de desbetreffende patiënt en op basis van de aanwezigheid van geïsoleerde of meerdere aangeboren afwijkingen. Gemiddeld werd er €26.627 per patiënt uitgegeven. 2,3% van deze kosten bestond uit kosten gerelateerd aan genetische kosten. De gemiddelde kosten voor patiënten met meerdere aangeboren afwijkingen (€53.686 per

patiënt) waren hoger dan de kosten voor patiënten met een geïsoleerde aangeboren afwijking (€27.350 per patiënt) en patiënten zonder aangeboren afwijking (€15.210 per patiënt). Vervolgens zijn er op basis van klinische preselectie vier verschillende scenario's gemodelleerd. Scenario 1, waarbij trio-ES de reguliere genetische diagnostiek vervangt voor alle patiënten, leidde tot een gemiddelde kostentoename van 22,2%. In het tweede scenario werd voor alle patiënten met meerdere aangeboren afwijkingen reguliere genetische diagnostiek vervangen door trio-ES, wat niet leidde tot veranderingen met betrekking tot de gemiddelde kosten per patiënt. Echter, patiënten met geïsoleerde aangeboren afwijkingen ontvangen in dit scenario geen genetische diagnostiek. Om deze reden is scenario 3 uitgevoerd, waarbij alle patiënten met een aangeboren afwijking, ongeacht of deze geïsoleerd was of niet, trio-ES ontvingen. Dit resulteerde in een kosten toename van 5,3%. Er zijn echter een aantal diagnosen die niet gedetecteerd kunnen worden met ES. Daarom is een vierde scenario gemodelleerd waarbij alternatieve genetische diagnostiek wordt ingezet om te compenseren voor de genetische diagnosen die anders gemist worden. Dit leidt tot een kostentoename van maximaal 5,5%. Uiteindelijk kan geconcludeerd worden dat genetische diagnostiek verantwoordelijk is voor een klein deel van de totale kosten. Implementatie van trio-ES voor alle patiënten met aangeboren afwijkingen leidt tot een beperkte toename in algehele kosten, terwijl het zorgt voor gerichtere zorg die beter is afgestemd op de patiënt op basis van de gestelde diagnose.

Ondanks dat klinische besluitvorming positief beïnvloed kan worden door de introductie van spoed ES bij ernstig zieke pasgeborenen die zijn opgenomen op de NICU, is er tot op heden beperkt prospectief onderzoek gedaan naar de impact van spoed ES ten opzichte van reguliere genetische diagnostiek. In **hoofdstuk 5** is een prospectieve klinische studie uitgevoerd, waarbij spoed ES is vergeleken met de huidige standaard diagnostiek. Vijf Nederlandse NICU's waren bij dit onderzoek betrokken en in totaal zijn 60 pasgeborenen met een verdenking op een genetische aandoening geïncludeerd. Elke patiënt ontving parallel aan elkaar, zowel spoed ES als reguliere genetische diagnostiek. Diagnostische opbrengst en de tijd tot diagnose werden onderzocht. De inzet van zorg werd in kaart gebracht om zodoende de economische impact van spoed ES te onderzoeken. Daarnaast werden vragenlijsten afgenomen bij ouders en clinici en werd de Visual Analogue Scale (VAS) score ingevuld om inzicht te krijgen in hun visie op spoed ES. In vergelijking met de reguliere genetische diagnostiek leidde spoed ES tot significant meer diagnosen (10% vs. 20% respectievelijk, $p<0,05$). Daarnaast was de tijd tot diagnose ook significant korter voor spoed ES (59 dagen (95% betrouwbaarheidsinterval 22-99) vs. 15 dagen (95% CI 10-20)). Voor de genetische diagnostische kosten leidde spoed ES tot een kosten besparing van 1,5% (€85 per patiënt). De voorkeur van ouders ging uit naar spoed ES vergeleken met reguliere genetische diagnostiek, en de betrokken clinici gaven aan dat spoed ES meer klinische voordelen heeft. De kostenbesparing en positieve gebruikerservaringen

rechtvaardigen de implementatie van spoed ES als eerste test die wordt ingezet bij ernstig zieke pasgeborenen met een verdenking van een genetische aandoening.

Conclusie

Dit proefschrift heeft aangetoond dat exoomsequencing zowel klinisch als economisch potentie heeft om onderdeel te worden van de reguliere genetische diagnostiek voor ernstig zieke kinderen die zijn opgenomen op de neonatale intensive care unit. Implementatie van exoomsequencing zal niet leiden tot een significante stijging van de kosten van de gezondheidszorg. Vooral niet wanneer deze technologie wordt geïmplementeerd als eerste diagnostische test voor alle pasgeborenen met een aangeboren afwijking. Met de implementatie van exoomsequencing is echter niet gewacht op de uitkomsten van de onderzoeken zoals gepresenteerd in dit proefschrift. Nu de technologische revolutie binnen de medische genetica nog in volle gang is, moeten toekomstige kosteneffectiviteitsanalyses eerder in het implementatieproces worden uitgevoerd. Op basis van vroege gezondheidseconomische evaluaties moeten beslissingen over implementatie gemaakt worden, bij voorkeur met behulp van valide en transparante beslissingsanalysemodellen die inzicht geven in toekomstige kosten en effecten. Vervolgens moeten deze modellen bijgewerkt worden zodra nieuwe gegevens uit de praktijk beschikbaar komen, zodat de desbetreffende technologie verder geïmplementeerd kan worden of besloten kan worden dat implementatie nooit kosteneffectief kan zijn en niet verder doorgezet wordt. Het modelleren houdt tevens in dat er onzekerheid wordt toegevoegd aan besluitvorming. Het gebruik van lange-termijn data van andere studies, in combinatie met kwalitatief hoogwaardige data uit de dagelijkse praktijk, kunnen er voor zorgen dat deze onzekerheid geminimaliseerd wordt en kan tijdig inzicht geven in de toegevoegde waarde van een technologie. Op deze manier kan er beter richting gegeven worden aan de toekomst van innovatieve diagnostische technologieën.

LIST OF PUBLICATIONS

Schobers G., Derks R., den Ouden A., Swinkels H., van Reeuwijk J., Bosgoed E., Lugtenberg D., Sun S.M., Corominas Galbany J., Weiss M., Blok M.J., **Olde Keizer R.A.C.M.**, Hofste T., Hellebrekers D., de Leeuw N., Stegmann A., Kamsteeg E.J., Paulussen A.D.C., Ligtenberg M.J.L., Bradley X.Z., Peden J., Gutierrez A., Pullen A., Payne T., Gilissen C., van den Wijngaard A., Brunner H.G., Nelen M., Yntema H.G., Vissers L.E.L.M. Genome sequencing as a generic diagnostic strategy for rare disease. Genome Med, 2024. 16(1):32.

Olde Keizer R.A.C.M.*, Marouane A.*, Kerstjens-Frederikse W.S., Deden A.C., Lichtenbelt K.D., Jonckers T., Vervoorn M., Vreeburg M., Henneman L., de Vries L.S., Sinke R.J., Pfundt R., Stevens S.J.C., Andriessen P., van Lingen R.A., Nelen M., Scheffer H., Stemkens D., Oosterwijk C., Ploos van Amstel J.K., de Boode W.P.*, van Zelst-Stams W.A.G.*, Frederix G.W.J.*, Vissers L.E.L.M.* on behalf of RADICON-NL consortium**. Rapid exome sequencing as first-tier test in neonates with suspected genetic disorder: results of a prospective multicenter clinical utility study in the Netherlands. European Journal of Pediatrics, 2023. 182(6): 2683-2692.

Olde Keizer R.A.C.M., Marouane A., Deden A.C., van Zelst-Stams W.A.G., de Boode W.P., Keusters W.R., Henneman L., Ploos van Amstel J.K., Frederix G.W.J.*, Vissers L.E.L.M.* Medical costs of children admitted to the neonatal intensive care unit: the role and possible economic impact of exome sequencing in early diagnosis. European Journal of Medical Genetics, 2022. 65(5): 104467.

Marouane A., **Olde Keizer R.A.C.M.**, Frederix G.W.J., Vissers L.E.L.M., de Boode W.P., van Zelst-Stams W.G.A. Congenital anomalies and genetic disorders in neonates and infants: a single-center observational cohort study`. European Journal of Pediatrics, 2022. 181(1): 359-367.

Olde Keizer R.A.C.M., Henneman L., Ploos van Amstel J.K., Vissers L.E.L.M., Frederix G.W.J. Economic evaluations of exome and genome sequencing in pediatric genetics: considerations towards a consensus strategy. Journal of Medical Economics, 2021. 24(Suppl 1): 60-70.

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ABOUT THE AUTHOR

Richelle A.C.M. Olde Keizer was born on the 4th of October 1992 in Oldenzaal, the Netherlands. After graduating from Twents Carmel College in Oldenzaal (gymnasium), she started the Bachelor Human Movement Sciences at the University of Groningen in 2010. During her Bachelor, Richelle focused her graduation project on the impact of cognitive impairment on risk management when crossing the street, upon which she received her Bachelor's degree in 2014.

Richelle continued to study the Master Human Movement Sciences, with a specialization in Rehabilitation. She did an internship at Reade in Amsterdam, where she performed research on the most effective treatment method for hand edema in patients with a high spinal cord injury. Afterwards, Richelle did two internships in 2015, both at Roessingh Research and Development in Enschede. These internships focused on the impact of perturbing balance of post-stroke patients on their gait pattern and on the correlation between clinical assessment scales and virtual stepping tasks on the C-Mill. After finishing these internships, Richelle received her Master's degree in 2016.

After working as a researcher at Roessing Research and Development in Enschede and TriviumMeulenbeltZorg in Almelo, Richelle started her PhD at the department of Heatlh Technology Assessment of the University Medical Center in Utrecht in 2017. The results of her PhD project are described in this thesis. During her PhD, Richelle studied the Master Clinical Epidemiology at Utrecht University, of which she obtained her Master's degree in 2021. From 2021-2023, Richelle worked at Radboud University Medical Center in Nijmegen, the Netherlands.

Since 2024, Richelle is working at Kennispunt Twente in Enschede, the Netherlands. Here, she can apply the knowledge she has obtained during the previous years.

In the last decade, the landscape of genetic testing has changed enormously due to the development and introduction of next-generation sequencing technologies. Exome sequencing has found a stable position in identifying the genetic etiology of rare disease, and increased diagnostic yield. There is however still an unmet need to better understand the economic impact of exome sequencing implementation in routine care, also including the other possible benefits of exome sequencing, such as improvements in clinical decision-making, and reduced burden to families. A cohort of patients in which these economic outcomes might be best captured are neonates admitted to Dutch neonatal intensive care units. For these neonates, it is expected that genetic tests results may impact clinical decision-making. Yet, genetic testing using exome sequencing has not reached widespread implementation. The latter is mainly due to the need for neonatal intensive care units to have a fast exome sequencing result, requiring infrastructure on Dutch genetic laboratories. There are also still uncertainties regarding the values to be taking into account for economic evaluation when implementing novel genetic tests. Hence, this thesis assesses the economic value of implementing exome sequencing compared to routine genetic diagnostic testing within pediatric care.

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