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Whole genome sequencing as a first-tier diagnostic test for infants in neonatal intensive care units: A pilot study in Brazil

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Abstract

In this pilot study, we aimed to evaluate the feasibility of whole genome sequencing (WGS) as a first-tier diagnostic test for infants hospitalized in neonatal intensive care units in the Brazilian healthcare system. The cohort presented here results from a joint collaboration between private and public hospitals in Brazil considering the initiative of a clinical laboratory to provide timely diagnosis for critically ill infants. We performed trio (proband and parents) WGS in 21 infants suspected of a genetic disease with an urgent need for diagnosis to guide medical care. Overall, the primary indication for genetic testing was dysmorphic syndromes ($n = 14$, 67%) followed by inborn errors of metabolism ($n = 6$, 29%) and skeletal dysplasias ($n = 1$, 5%). The diagnostic yield in our cohort was 57% (12/21) based on cases that received a definitive or likely definitive diagnostic result from WGS analysis. A total of 16 pathogenic/likely pathogenic variants and 10 variants of unknown significance were detected, and in most cases inherited from an unaffected parent. In addition, the reported variants were of different types, but mainly missense (58%) and associated with autosomal diseases (19/26); only three were associated with X-linked diseases, detected in hemizygosity in the proband an inherited from an unaffected mother. Notably, we identified 10 novel variants, absent from public genomic databases, in our cohort. Considering the entire diagnostic process, the average turnaround time from enrollment to medical report in our study was 53 days. Our findings demonstrate the remarkable utility of WGS as a diagnostic tool, elevating the potential of transformative impact since it outperforms conventional genetic tests. Here, we address the main challenges associated with implementing WGS in the medical care system in Brazil, as well as discuss the potential benefits and limitations of WGS as a diagnostic tool in the neonatal care setting.

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KEYWORDS
clinical pediatrics, diagnostic yield, genetic diseases, next-generation sequencing (NGS), trio testing, whole genome sequencing

1 | INTRODUCTION

Genetic diseases and congenital anomalies occur in ~6% of all live births and represent the leading cause of infant hospitalization in neonatal intensive care units (NICU; Hagen et al., 2022; Marouane et al., 2022; Swaggart et al., 2019). In particular, congenital anomalies, possibly indicating an underlying genetic disease, account for 14% of all admissions to NICU and it is the primary reason for mortality among newborns (Hudome et al., 1994; Jacob et al., 2015; Stevenson & Carey, 2004; Synnes et al., 2004; Weiner et al., 2011). Nonetheless, providing early etiological diagnosis for critically ill infants, which is crucial for optimizing clinical outcomes, is exceptionally challenging due to the vast number of over 8000 genetic diseases, often presenting atypical symptoms (Boycott & Ardigó, 2018; Hartley et al., 2018). Although the shift from empirical treatment to accurate clinical management of identified genetic diseases is facilitated by timely diagnosis, the slow turnaround time of genetic testing results limited their real-time application in critical care medicine. In such complex and critical scenario, whole genome sequencing (WGS) has been recommended as a first-tier diagnostic test for infants admitted to NICU (Bowling et al., 2022; Denommé-Pichon et al., 2022; Farnaes et al., 2018; French et al., 2019; Hayeems et al., 2017; Sanford et al., 2019; Sanford Kobayashi et al., 2022). Notably, despite its initial high cost, WGS has the potential to decrease overall expenses, especially in terms of hospital length of stay (Chung et al., 2020; Farnaes et al., 2018; Sanford Kobayashi et al., 2022). This can be attributed to its ability to provide more comprehensive genetic information, which can help shorten the stressful and emotionally burdensome diagnostic odyssey experienced by families. Neonatologists have progressively recognized the benefit of WGS since it helps mitigate the rapid pace of discovery in genomics, considering the estimated 250 new gene-phenotype associations being discovered each year (Bamshad et al., 2019; Kingsmore et al., 2019; Palmquist et al., 2022; Saunders et al., 2012).

The genome sequencing of the proband and their parents (trio), when possible, reduces the number of candidate variants and improves diagnostic accuracy. It is worth mentioning that, traditionally, genetic tests are used stepwise to identify disease-causing variants in Brazil, starting with karyotype, then microarray, and eventually progressing to exome sequencing. However, access to these tests can be difficult in low- and middle-income countries. Exome or genome sequencing is not often covered by the Brazilian Unified Health System (Sistema Único de Saúde-SUS). Karyotype and microarray must be performed, and a negative result must be confirmed before exome sequencing can be ordered and covered by the patient's private health insurance. While many programs worldwide have shown that WGS improves clinical outcomes and reduces costs in the NICU setting (Chung et al., 2020; Farnaes et al., 2018; Sanford Kobayashi et al., 2022), implementing such a test within a national healthcare system poses significant challenges. Among others, these include balancing the extreme stress of families with the complexities of the informed consenting process, incomplete understanding of new technology leading to uncertainty for healthcare providers, and a lack of consensus on reporting secondary findings. In this pilot study, we aimed to evaluate the clinical utility of WGS as a first-tier diagnostic test for infants hospitalized in NICU in Brazil. Our study was designed to assess the feasibility of improving the speed and accuracy of molecular diagnosis for critically ill infants and to address the main challenges associated with the current diagnostic process in our medical system.

2 | PATIENTS AND METHODS

2.1 | Study design

This is a prospective, multicenter, observational study that enrolled 21 hospitalized infants in NICU from private and public hospitals in Brazil between March 2022 and September 2022. Three clinical sites participated in the research, namely Instituto da Criança – FMUSP (São Paulo, SP), Hospital Infantil Sabará (São Paulo, SP), and Hospital das Clínicas de Porto Alegre – HCPA (Porto Alegre, RS). The study was approved by the Ethics Committee from Hospital Moriah (CAAE: 44497221.6.0000.8054) and conducted in accordance with ethical principles established in the Declaration of Helsinki, and Resolution 466/2012 of the Brazilian National Health Council. Patient's parents were required to provide written informed consent to participate in the research. Clinical information was collected from the genetic testing requisition form, which was filled out by the physicians, and included: sex, age, phenotype, and opt-in to receive secondary findings. Parents' WGS results were used for the probands' WGS data interpretation. Figure 1 shows a flowchart of the present study. All relevant clinical and molecular information from this pilot study is presented in Table S1.

2.2 | Enrolled infants

We enrolled infants referred due to the following conditions: (1) infants hospitalized in NICU suspected of a genetic disease and no apparent clinical diagnosis; (2) urgent need for an etiological diagnosis to guide medical care; (3) proband and both biological parents available for blood samples; and (4) informed consent signed by both parents. The inclusion criteria were validated by the clinical geneticist coordinating the project. After medical consultation, blood samples were collected from the proband and their parents at the clinical sites.

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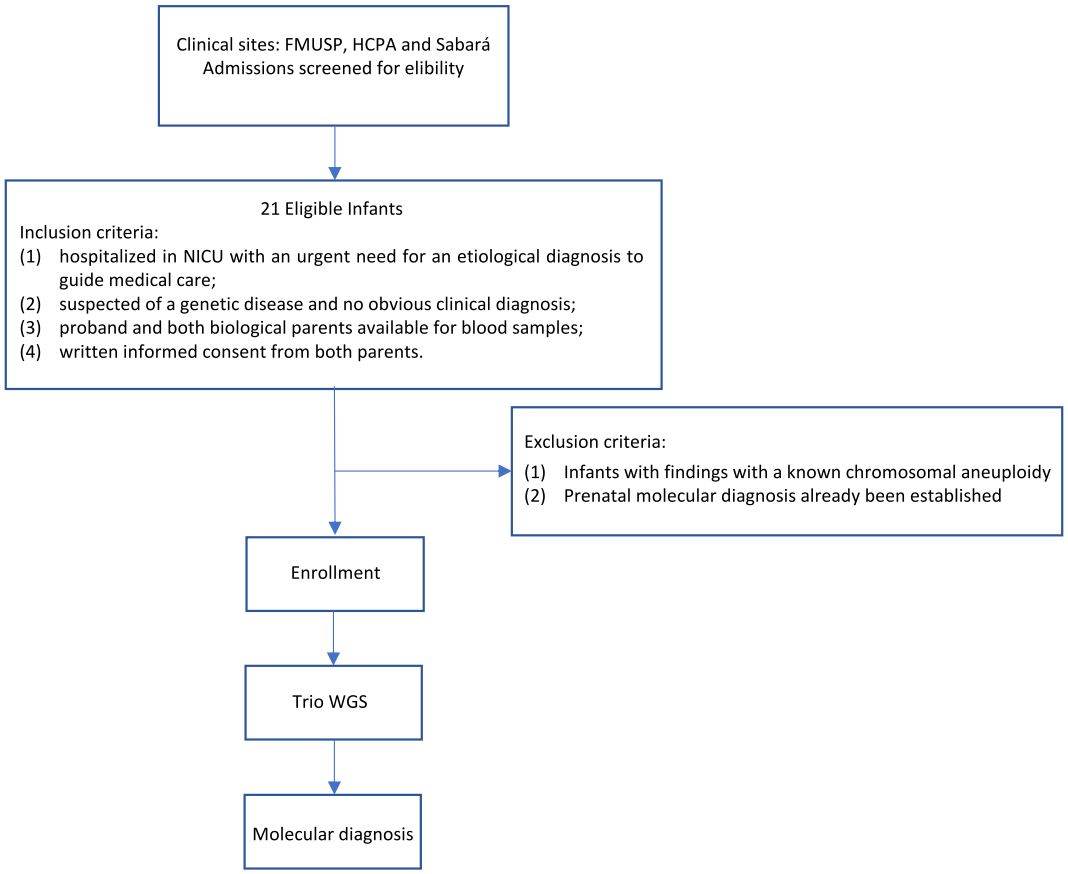

FIGURE 1 Flowchart of this pilot study. A total of 21 infants suspected of a genetic disease and hospitalized in neonatal intensive care units (NICU) were enrolled. Three clinical sites participated in this research: Instituto da Criança – FMUSP (São Paulo, SP), Hospital Infantil Sabará (São Paulo, SP), and Hospital das Clínicas de Porto Alegre – HCPA (Porto Alegre, RS). Whole genome sequencing (WGS) analysis includes collecting trio (proband and both parents) for molecular diagnosis.

Infants were excluded from the study if they had findings consistent with a known chromosomal aneuploidy (e.g., trisomy 13, 18, and 21 and monosomy X), or if they had a prenatal molecular diagnosis already established.

2.3 | Whole genome sequencing

Peripheral blood samples collected in EDTA tubes were sent to DASA Genomic laboratory (São Paulo, SP) for DNA extraction (QIAsymphony DSP DNA Mini Kit), storage, and sequencing experiments. WGS from the probands and their parents was performed for the detection of single nucleotide variants (SNVs), small insertions and deletions (InDels), and copy number variations (CNVs). According to the manufacturer's recommendation, DNA libraries were built using the Illumina TruSeq DNA PCR Free kit (California, USA). DNA library fragments were sequenced from both ends (paired) with a read length of 150 base pairs using the Illumina NovaSeq 6000 platform (California, USA). The targeted mean coverage depth was 30× with >80% of bases covered at 20×. Sequence reads were aligned to the GRCh38 reference genome utilizing the DRAGEN Germline App v3.9.5 (Illumina, Inc., California, USA). The same platform was used for processing quality control (QC) metrics and variant calling. The resolution of CNVs in our analysis was >30 kb. Other structural variants were not assessed in this study. The QC metrics and analytical performance of WGS data are presented in Tables S2 and S3, respectively.

2.4 | Annotation, filtering, and variant classification

Sequence data were analyzed using the Emedgene platform (Illumina, Inc., California, USA) and the PhenoDB Variant Analysis Tool (Sobreira et al., 2015). VCF files were used for annotation and filtering of genetic variants. Visual verification of the findings was made using data from the BAM files. Variant analysis and interpretation were conducted as part of a research protocol. As part of our filtering strategy, we selected: (1) variants with minor allelic frequency <1% (1000 Genomes; dbSNP; dbVar; gnomAD); (2) variants in disease-causing genes per MIM; (3) exonic and splicing variants; (4) variants not located in segmental duplications (excluding pseudogenes). The selected variants were classified as pathogenic (P), likely pathogenic

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(LP), benign (B), likely benign (LB), or variants of unknown significance (VUS) according to the *American College of Medical Genetics and Genomics* (ACMG) guidelines (Richards et al., 2015). We tested all modes of inheritance, starting with autosomal dominant hypothesis (investigation of de novo variants), followed by autosomal recessive or X-linked (homozygosity, compound heterozygosis, and hemizygosity). Sanger sequencing was used to validate variants suspected of false positives, that is, variants with a vertical coverage <20× or when an allele balance bias was observed.

2.5 | Case classification

Gene selection and variant interpretation were guided through the clinical history of the patient and Human Phenotype Ontology (HPO) terms. The selected variants were assigned a case-level classification that linked the variant to confidence in causation. The variants were classified as definitive diagnostic (DD), likely diagnostic (LD), or inconclusive (IN). Most infants harboring pathogenic or likely pathogenic variants received a case-level classification of DD. However, for a few cases, a pathogenic or likely pathogenic variant received a case-level classification of IN because (1) the zygosity of the variant did not follow the expected mode of inheritance associated with the disease caused by the gene (only one heterozygous variant was identified in a gene associated with an autosomal recessive condition); (2) the probands' phenotype did not overlap with the phenotype caused by pathogenic variants in the gene; or (3) we could not define if the variants were in *cis* or *trans*. In contrast, some probands that harbored a VUS received a case-level classification of LD when it was identified in *trans* with another P/LP variant. This association was particularly explored in genes, when altered, cause a phenotype consistent with the clinical presentation of the patient.

2.6 | Return of results and limitations

One final genome report was delivered for each trio since genomic data from the parents were used exclusively to aid in the analysis of the probands' WGS data. Only variants classified as pathogenic, likely pathogenic, or VUS in a gene known to cause a disease related to the probands' phenotype were reported. Secondary findings were reported when the probands' parents opted-in as indicated in the informed consent. In this case, only variants classified as pathogenic or likely pathogenic in AMG actionable genes were reported (Miller et al., 2022). WGS results were delivered to the physician at the clinical sites, and each hospital was responsible for guiding medical care and providing proper genetic counseling to the family. Of note, the bioinformatics pipeline utilized in this study was optimized for constitutive variants, so mosaicism cases may not be detected. A limited sensitivity is also expected on genes with high homology (i.e., *SMN1/SMN2*, *HBA1/HBA2*, *CYP21A2/CYP21A1P*, *PMS2/PM2CL*, *GBA/GBAP1*) due to poor mapping quality of short reads on regions with high similarity.

3 | RESULTS

3.1 | Demographic and clinical features

We performed trio WGS in 21 hospitalized infants over a period of 7 months. Although most patients were referred from public hospitals in Brazil (HC-USP and HCPA), it is worth mentioning that the private hospital Sabará was the last clinical site to participate in this research, thus, had a lower recruitment rate. Age range was from 12 to 180 days (median age: 28 days) at the time of enrollment, and 86% ($n = 18$) of the patients were male. Overall, the primary indication for genetic testing was dysmorphic syndromes ($n = 14$; 67%) followed by inborn errors of metabolism ($n = 6$; 29%) and skeletal dysplasias ($n = 1$; 5%; Table 1).

TABLE 1 Demographic and clinical characteristics of the infants enrolled in this pilot study.

Characteristics	Total *n* (%)	Patients with DD/LD result *n* (%)	Patients with IN result *n* (%)
Clinical sites			
HC-USP	11 (52%)	6 (50%)	5 (56%)
HCPA	7 (33%)	5 (42%)	2 (22%)
Hospital Sabará	3 (14%)	1 (8%)	2 (22%)
Sex			
Male	18 (86%)	10 (83%)	8 (89%)
Female	3 (14%)	2 (12%)	1 (11%)
Age, median (range)			
Age of proband at enrollment (days)	28 (12–180)	-	-
Primary indication for genetic testing			
Dysmorphological syndromes	14 (67%)	7 (58%)	7 (78%)
Inborn errors of metabolism	6 (29%)	4 (33%)	2 (22%)
Skeletal dysplasias	1 (5%)	1 (8%)	-

Abbreviations: DD/LD, definitive diagnostic/likely diagnostic; IN, inconclusive.

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With input from medical geneticists, we documented patient clinical features using HPO terms, which were grouped into 12 relevant phenotypic categories (Table S4). These categories include a total of 45 HPO terms. Notably, all patients presented phenotypic features corresponding to more than one category as seen in Table SS1.

Figure 2a summarizes the most frequent clinical features encountered in our cohort. The top 15 HPO terms comprise cardiac malformations ($n = 6$; 13%), failure to thrive ($n = 6$; 13%), dysmorphisms ($n = 5$; 11%), premature birth ($n = 5$; 11%), congenital diaphragmatic hernia ($n = 3$; 7%), hepatosplenomegaly ($n = 3$; 7%), hypoglycemia ($n = 3$; 7%), macrocephaly ($n = 3$; 7%), seizure ($n = 3$; 7%), adrenal hyperplasia ($n = 2$; 4%), ambiguous genitalia ($n = 2$; 4%), hypertrophic cardiomyopathy ($n = 2$; 4%), hypotonia ($n = 2$; 4%), metabolic acidosis ($n = 2$; 4%), and recurrent infections ($n = 2$; 4%).

3.2 | Diagnostic yield

Out of the 21 infants enrolled in this study, 12 received a DD/LD result, reflecting an overall diagnostic yield of 57% in our cohort. The remaining nine patients (43%) received an IN result either because they harbored a VUS or no pathogenic/likely pathogenic variants were detected. In most cases ($n = 8$; 38%), the disease-causing variant was inherited from the unaffected parent whereas in 19% of cases ($n = 4$) the variant occurred de novo (Figure 2b). All families consented to receive secondary findings in the ACMG actionable genes (Miller et al., 2022) but only one pathogenic variant was detected in one infant. Turnaround time from enrollment to medical report averaged 53 days; an overview of the diagnostic process in this pilot study is presented in Figure S1.

3.3 | Characteristics of the disease-causing variants

Overall, a total of 16 disease-causing variants (P/LP) and 10 VUS were identified across 17 MIM genes. Ten novel variants, not present in any public genomic database, were observed in our cohort. In most of the DD/LD cases (65%), the infants inherited compound heterozygous or homozygous variants in a gene associated with an autosomal

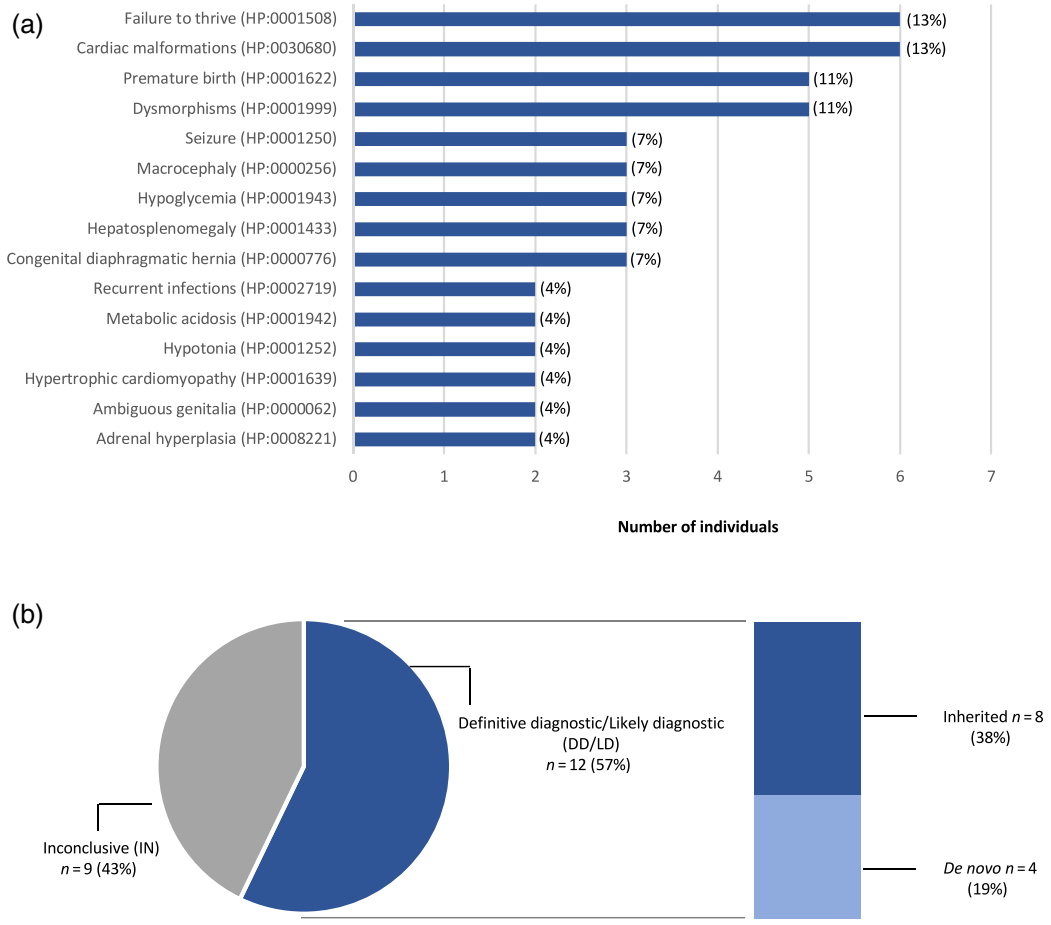

FIGURE 2 Phenotypic characterization and diagnostic yield of whole genome sequencing in our cohort. (a) Frequency distribution of the most common Human Phenotype Ontology (HPO) terms encountered in our cohort. The horizontal bar chart displays the top 15 HPO terms. (b) The pie chart displays the overall diagnostic yield based on cases that received a definitive or likely definitive diagnostic (DD/LD) or an inconclusive (IN) result from sequencing. On the right side the bar chart show that in most cases the disease-causing variant was inherited from the parent.

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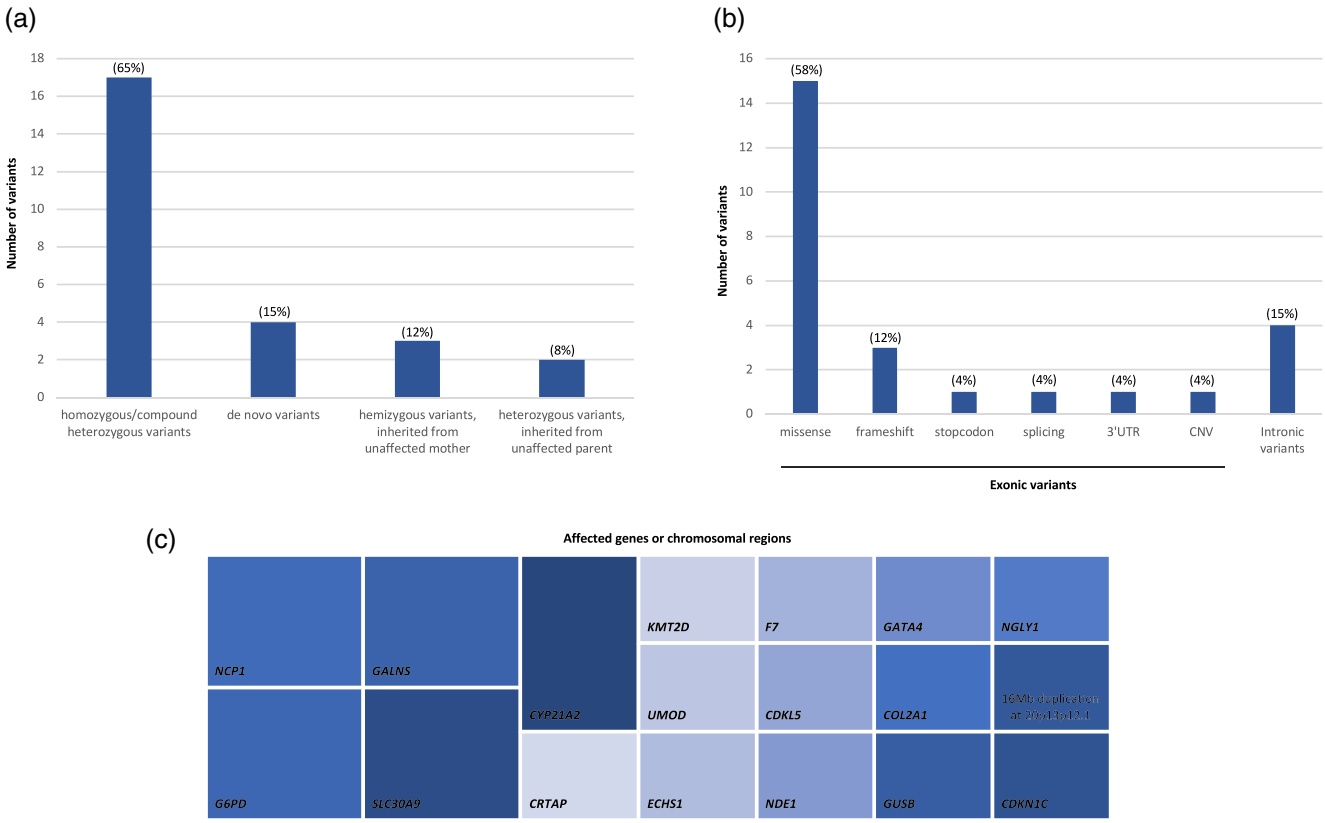

FIGURE 3 Characteristic of the disease-causing variants identified from whole genome sequencing analysis in our cohort. (a) Frequency distribution of all disease-causing variants based on the inheritance mode. (b) All returnable variants representing different types of variations with missense variants being the most frequent. (c) Spectrum of the disease-causing variants listing the affected genes or chromosomal regions.

recessive disease ($n = 17/26$), whereas in 15% ($n = 4/26$) the infants inherited a de novo heterozygous variant in a gene known to cause an autosomal disease. An additional 12% ($n = 3/26$) represent hemizygous variant, in males, inherited from unaffected mother, and 8% ($n = 2/26$) correspond to heterozygous variant inherited from unaffected parent (Figure 3a). Furthermore, the reported variants represent different types of variations, being most of them exonic; 58% of the disease-causing variants result in missense, 12% in frameshift, and 4% in stopcodon. Also, 4% of them were either predicted to disrupt splicing or present in 3′ Untranslated Region (UTR), and there was one case resulting from a CNV, a 16 Mb duplication at 20p13p12.1 (Figure 3b). The spectrum of the disease-causing variants is shown in Figure 3c. Table 2 presents a summary of all variants identified in this study, including the associate disorder, and the novel variants highlighted in bold. The total number of P/LP variants and VUS detected in our cohort was 18 and 17, respectively, considering both the IN and DD/LD cases.

4 | DISCUSSION

Several studies have shown the remarkable clinical utility of WGS to shorten the diagnostic process for critically ill infants. Here, we report our experience implementing WGS as a first-tier diagnostic test for infants hospitalized in the NICU in the Brazilian healthcare system. The patients were referred from public and private hospitals in Brazil in order to gather a more comprehensive perspective on the application of WGS as diagnostic tool in the neonatal care setting; understanding the unique challenges and opportunities of implementing WGS in a country with distinct healthcare resources and infrastructure was essential to evaluate its potential impact on clinical practice and patient care.

By employing trio WGS in 21 infants suspected of a genetic disease we achieved an overall diagnostic yield of 57%. Our results are in accordance with previous publications from other groups and show the robustness and effectiveness of our approach to provide timely diagnosis for critically ill infants. The reported causative variants included SNVs, InDels, and CNVs, but missense variants were the most common (58%), in agreement with prior WGS studies. Further, autosomal recessive was the most common mode of inheritance, with infants harboring compound heterozygous or homozygous variants in a gene known to cause an autosomal recessive disease. There were no recurrent diagnoses, affected genes, or chromosomal regions, reflecting genetic heterogeneity in critically ill infants. Notably, 10 novel variants were observed in our cohort. This expressive number in a small cohort may reflect the underrepresentation of the Brazilian population in public genomic databases. Regarding the phenotypes, dysmorphological syndromes represent the primary indication for genetic testing (67%), but stratification of specific HPO terms

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TABLE 2 All detected variants identified from whole genome sequencing data analysis in our cohort.

ID	Affected gene(s) or chromosomal segment	HGVS nomenclature	Associated disorder	(MIM) gene	Inheritance pattern	Variant classification	Case classification	TAT (days)
1	-	-	-	-	-	-	IN	21
2	*GLA*	NM_000169.3: c.1183G > C	Fabry disease	300,644	XL	VUS	IN	41
	METTL23	NM_001080510.4: c.174_177del	Intellectual developmental disorder, autosomal recessive 44	615,262	AR	P		
3	*NADSYN1*	NM_018161.5: c.1477G > A	Vertebral, cardiac, renal, and limb defects syndrome 3	608,285	AR	VUS	IN	37
		NM_018161.5: c.1562 + 1675G > T				VUS		
4	-	-	-	-	-	-	IN	53
5	*CRTAP*	NM_006371.5: c.320_321del	Osteogenesis imperfecta type VII	5497	AR	P	DD	19
		NM_006371.5: c.155 T > C				VUS		
6	-	-	-	-	-	-	IN	44
7	*KMT2D*	**NM_003482.3: c.8542C > T**	Kabuki syndrome	602,113	AD	P	DD	63
	UMOD	**NM_003361.3: c.362A > G**	Tubulointerstitial kidney disease autosomal dominant type 1	191,845	AD	VUS		
8	*SNIP1*	NM_024700.4: c.346C > T	Neurodevelopmental disorder with hypotonia, craniofacial abnormalities, and seizures	608,241	AR	VUS	IN	39
		NM_024700.4: c.926 + 867dup				VUS		
9	*ECHS1*	NM_004092.4: c.476A > G	Mitochondrial short-chain enoyl-CoA hydratase 1 deficiency	616,277	AR	P	DD	39
		NM_004092.4 c.583G > A				LP		
10	*F7*	**NM_000131.4: c.1303G > A**	Factor VII deficiency	613,878	AR	LP	LD	36
	CDKL5	NM_003159.2: c.2183A > G	Developmental and epileptic encephalopathy 2	300,203	XLD	VUS		
	NDE1	NM_001143979.2: c.872C > T	Lissencephaly 4 (with microcephaly) and Microhydranencephaly	609,449	AR	VUS		
11	*GATA4*	**NM_002052.5: c.722G > A**	Testicular anomalies with or without congenital heart disease/Atrial septal defect type 2/Atrioventricular septal defect type 4/Tetralogy of Fallot/Ventricular septal defect type 1	576	all AD	LP	LD	30
12	13 kb duplication at Xp11.23	**NC_000023.11: g.48790364_48803945dup**	-	-	-	VUS	IN	48
13	*NGLY1*	NM_018297.4: c.1004-221C > T	Congenital disorder of deglycosylation type 1	610,661	AR	VUS	LD	26
		NM_018297.4: c.*115 T > C				VUS		

(Continues)

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TABLE 2 (Continued)

ID	Affected gene(s) or chromosomal segment	HGVS nomenclature	Associated disorder	(MIM) gene	Inheritance pattern	Variant classification	Case classification	TAT (days)
14	*COL2A1*	**NM_001844.5: c.905C > T**	Multiple phenotypes (-Epiphyseal dysplasia, multiple, with myopia and deafness/-Vitreoretinopathy with phalangeal epiphyseal dysplasia/-Achondrogenesis, type II or hypochondrogenesis/-Avascular necrosis of the femoral head/-Czech dysplasia/-Kniest dysplasia/-Legg–Calve–Perthes disease/-Osteoarthritis with mild chondrodysplasia/-Platyspondylic skeletal dysplasia, Torrance type/-SED congenita/-SMED Strudwick typ/-Spondyloepiphyseal dysplasia, Stanescu type/-Spondyloperipheral dysplasia/-Stickler syndrome, type I/-Stickler syndrome, type I, nonsyndromic ocular	120,140	all AD	P	DD	46
	NPC1	NM_000271.5: c.3104C > T	Niemann–Pick disease, type C1 and D	7623	AR	LP		
		NM_000271.5: c.288-1011G > A		7623	AR	VUS		
15	132 kb deletion at 1q43	NC_000001.11: g.243199772_243332454del	-	-	-	VUS	IN	49
16	*G6PD*	NM_001360016.2:c.376A > G	Hemolytic anemia, G6PD deficient (favism)	305,900	XL	LP	LD	63
		NM_001360016.2:c.968 T > C				LP		
	GALNS	NM_000512.5: c.499 T > G	Mucopolysaccharidosis IVA	612,222	AR	LP		
	GALNS	NM_000512.5: c.423-901_423–900 delinsCT	Mucopolysaccharidosis IVA	612,222	AR	VUS		
17	*KIAA0586*	NM_001244189.2: c.428del	Joubert syndrome 23 and Short-rib thoracic dysplasia 14 with polydactyly	610,178	AR	P	IN	90
18	*GUSB*	NM_000181.4: c.307C > T	Mucopolysaccharidosis VII	611,499	AR	LP	DD	90
19	16 Mb duplication at 20p13p12.1	NC_000020.11: g.61182_16804071dup	-	-	-	P	DD	62
20	*CDKN1C*	**NM_000076.2: c.820 + 1G > C**	Beckwith–Wiedemann syndrome and IMAGE syndrome	600,856	AD	LP	DD	69
	SLC30A9	NM_006345.4: c.40del	Birk–Landau–Perez syndrome	604,604	AR	LP		
		NM_006345.4: c.528-28A > G		604,604	AR	VUS		
21	*CYP21A2*	NM_000500.9: c.955C > T	Adrenal hyperplasia, congenital, due to 21-hydroxylase deficiency and Hyperandrogenism, nonclassic type, due to 21-hydroxylase deficiency	613,815	AR	P	LD	150
		NM_000500.9: c.1450dup				VUS		

Abbreviations: AD, autosomal dominant; AR, autosomal recessive; DD, definitive diagnostic; ID, identification; IN, inconclusive; LD, likely diagnostic; LP, likely pathogenic; P, pathogenic; TAT, turnaround time; VUS, variant of unknown significance; XL, X-linked; XLD, X-linked dominant.

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among congenital anomalies and its correlation with the likelihood of receiving a DD/LD result was not possible to be made in this study. It is relevant to mention that recent advances in high-throughput sequencing technologies combined with a phenotype-driven analysis have made rapid molecular diagnosis a viable option. Saunders et al. (2012) were the first to describe, in a proof-of-concept study, a 50-h molecular diagnosis of genetic diseases using WGS which was designed for use in NICU (Saunders et al., 2012). However, the term "rapid" is not universal in the literature and the median time to diagnosis using WGS ranges from 1 to 43 days. This difference depends on the country, resources, year of the study, and so forth. In this pilot study, considering the entire diagnostic process, the average turnaround time from enrollment to medical report was 53 days. Hence, WGS significantly surpassed the conventional genetic testing protocol encompassing karyotype, microarray, and exome sequencing, which exhibited an average turnaround time of 95 days.

Despite the successful diagnostic rate presented here it is important to highlight the challenges and limitations encountered during our study to provide valuable insights into the practical implementation and interpretation of WGS results. One of the main reasons the proband could not be enrolled in the study was the lack of parental samples. The lack of detailed phenotypic description was another challenge and limited the researchers' ability to comprehensively evaluate the sequencing data and make accurate diagnoses. We were also unable to have electronical medical records to collect the complete clinical history of the proband, which is crucial to access and integrate patient information, potentially impacting the depth of analysis. Finally, the cost of the WGS is still a limitation for the implementation of this test in the Brazilian healthcare system. To overcome all these challenges and limitations, we suggest a collaborative approach that leverages shared sequencing facilities. By collaborating, multiple institutions and laboratories can pool resources and expertise, leading to lower costs and faster turnaround times. Moreover, the establishment of a strong collaboration between academia, industry, and governmental organizations can potentially generate financial support for research projects. These partnerships may empower the development of cutting-edge genomic technologies and enable the development as well as application of new genetic tests. We also understand that encouraging studies that evaluate the economic impact of WGS in our healthcare system would be beneficial. Demonstrating the cost-effectiveness and clinical utility of WGS will help garner support from private health insurance companies, leading to increased coverage and accessibility for patients. Nonetheless, it is important to characterize social indicators that predict health disparities regarding access to genomic sequencing. Identifying and addressing these indicators is important to promote equity in our healthcare system and ensure that individuals from all socioeconomic backgrounds have equal opportunities on the access of genetic tests. This may involve targeted initiatives, health policies, or educational programs to bridge the gap and promote equal access to genetic services (Fishler et al., 2022).

In conclusion, the healthcare system in Brazil poses distinctive challenges in the integration and implementation of new health policies. However, we demonstrated the remarkable clinical utility of WGS as a diagnostic tool in our neonatal care setting, highlighting its transformative impact in patient clinical management. Our results show the precise nature of WGS, reinforcing its application as a first-tier diagnostic tool for neonates in the NICU. The implementation of WGS improves patient care, treatment efficacy, and reproductive counseling for these vulnerable patients and their families. As a result, we urge public agencies in Brazil to earnestly consider the integration of WGS into their healthcare framework.

AUTHOR CONTRIBUTIONS

Conceptualization: M.P.M., N.S. *Methodology*: N.H., R.G.C., W.B.C., R.S.H., D.R.B., L.S., R.S.P., G.L.Y., C.A.K., R.C.S., and R.G. *Software*: L.S., C.A.S., J.S.S., J.E.K., M.B., and R.G-S.; *Validation*: L.S., C.A.S., J.S.S., J.E.K., M.B., and R.G-S. *Formal analysis*: M.P.M., J.S., D.B., M.G., and D.A. *Data curation*: M.P.M., J.S., D.B., M.G., and D.A. *Visualization*: D.V. *Writing—review and editing*: M.P.M., D.V., C.R., and N.S. *Supervision*: N.S. *Project administration*: M.P.M. and F.M. *Funding acquisition*: C.S.-N. and G.A.C.

CONFLICT OF INTEREST STATEMENT

J.S., G.L.Y., R.G., and N.S. declare that they served as consultant for DASA. All other authors report no conflict of interest relevant to this article.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions. All data generated or analyzed during this study are included in this published article [and its supplementary information files].

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