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Availability of Genetic Tests in Public Health Services in Brazil: Data from the Brazilian Rare Diseases Network

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Keywords
Genetic tests · Public health · Rare diseases · Specialized centers · Brazilian public health system

Abstract
Introduction: The Brazilian Policy for Comprehensive Care for People with Rare Diseases (BPCCPRD) was published in 2014, accrediting several reference centers and incorporating many genetic tests for the diagnosis of rare diseases (RDs). The Brazilian Network of Rare Diseases (RARAS) comprises more than 40 institutions that offer diagnosis and treatment for RDs in Brazil. This network includes Reference Services for Rare Diseases (RDRS), Reference Services for Newborn Screening (NSRS), and University Hospitals distributed in all Brazilian regions. ***Objective:*** The aim of the study was to map the availability and distribution of the BPCCPRD diagnostic procedures in the Brazilian Unified Health System through RARAS. ***Method:*** Data were collected through a questionnaire on the Research Electronic Data Capture platform, with 22 questions regarding the availability of procedures. Thirty-seven coordinators from RARAS participating centers received the questionnaire link for participation by email from August/2020 to March/2021. All participating institutions ethically approved this project. ***Results:*** Of the 37 institutions, 23 (62.16%) offered cytogenetic tests, 20 (54.05%) offered molecular procedures, and 22 (59.46%) offered inborn errors of metabolism diagnostic tests. The Southern blot analysis, enzyme assays on cultured tissue and urinary organic acid tests had the highest outsourcing rate. On the other hand, the procedures most frequently performed on-site were bone marrow karyotype and long-term cultured karyotype. It was observed that 10 of the 37 centers (27%) did not provide access to investigated procedures (on-site or outsourced). The North and Midwest regions stood out in terms of the unavailability of such techniques in at least 40% of the evaluated institutions. ***Discussion and Conclusion:*** This study reveals large discrepancies in the supply of diagnostic procedures in the Brazilian territory. Moreover, there is a broad collaboration between services through the outsourcing of multiple diagnostic techniques to address this issue. Finally, this work corroborates the importance of mapping services for the diagnosis and treatment of individuals with RDs to propose actions for the better supply and distribution of these procedures.

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Introduction

A rare disease (RD) is any condition that affects up to 65 individuals per 100,000, according to the World Health Organization (WHO). Although individually rare, these conditions collectively affect up to 8% of the population and significantly impact the health system as they are generally considered chronic and disabling [1]. Therefore, health professionals must have consistent knowledge of their diagnosis, management, and treatment [2, 3]. It is estimated that 80% of the known RDs have a genetic etiology [4].

Brazil is the fifth largest country in the world, with an area of 8,510,345 km^2 and an estimated population of 214 million people. The country is organized into five geographic regions (North, Northeast, Midwest, Southeast, and South), 26 states (one federal district), and 5,570 municipalities [5].

The Unified Health System (SUS: Sistema Único de Saúde) guarantees universal and equal access to the promotion, protection, and restoration of health for all citizens at no cost to users in Brazil since 1988 [6, 7]. In recent decades, Brazil has experienced an epidemiological transition, with a significant improvement in health indicators due to the control of diseases caused by malnutrition, sanitary conditions, and external pathogens. Hereditary diseases and congenital anomalies are responsible for an increasing proportion of child deaths and are, since 2005, the second most common cause of infant mortality in all Brazilian regions [6, 7].

Until 2014, RDs were diagnosed and treated in specialized centers such as medical genetics services in University Hospitals (UH) or Newborn Screening Referral Services (NSRS). These centers were located mainly in large cities and state capitals and offered RD diagnosis through clinical and especially research laboratories as most genetic tests were not offered by the Unified Health System [8] and the only available test was conventional karyotype. According to Melo and Sequeiros [6], only a small segment of the population had access to molecular tests via public health assistance, usually through UH where the test was often offered in the context of care-associated research [6, 8], through judicialization [9] or through support networks that can enhance access to diagnostic tests [8, 10]. According to previous studies, the availability of diagnostic tests for genetic diseases followed a similar distribution to that of medical genetics services in Brazil, with a greater concentration in the South and Southeast regions [7, 11].

Although the number of private genetics laboratories has been expanding in Brazil, most clinical services in medical genetics are concentrated in university or public hospitals in large urban centers. Furthermore, public and academic institutions' are not always equipped with laboratories and personnel to provide the genetic tests needed for diagnosis [6, 7, 912, 13].

In January 2014, the Ministry of Health implemented the Brazilian Policy for Comprehensive Care for People with Rare Diseases (BPCCPRD). The objective of this policy was to reduce mortality, contribute to the reduction of morbidity and secondary manifestations, and improve the quality of life of people with RDs through health promotion, prevention, early detection, the provision of opportunities for treatment, the reduction of disability, and the promotion of palliative care [14].

The organization of care for people with RDs in Brazil is structured along two main axes: (i) RDs of genetic origin, including congenital anomalies and late-onset disorders (I-1), intellectual disability (I-2), and inborn errors of metabolism (IEM, I-3) and (ii) RDs of non-genetic origin, including infectious, inflammatory, and autoimmune diseases [14]. Since the publication of the policy, 21 Reference Services for Rare Diseases (RDRS), responsible for the diagnosis and treatment of RDs [14], have been implemented (online supplemental Material; for all online suppl. material, see https://doi.org/10.1159/000541547). Considering the Brazilian territory and population, RD care facilities are insufficient for the population size. There are still considerable barriers to accessing care, including shortages of trained professionals and reference centers to treat these patients and difficulty in obtaining early and accurate diagnoses. These barriers lead to delayed diagnoses and limited access to diagnostic and therapeutic resources [4, 15].

The same ordinance that instituted the BPCCPRD in 2014 established financial incentives for the diagnosis of RDs, including biochemical and genetic analyses in the list of tests covered by the SUS, carried out by the rare diseases services [14]. Initially, the BPCCPRD incorporated specific diagnostic procedures for RDs of genetic origin (axes I-1, I-2, and I-3) [14]; later, at the end of 2020, whole-exome sequencing (WES) was included for the diagnosis of intellectual disability of unknown cause (Table 1). However, there are no incentives for laboratory facilities and the tests can be performed at the center or outsourced to a different laboratory. Among the procedures foreseen by the policy, there is no provision for diagnostic procedures related to axis 2 (non-genetic RDs). The provision of financial incentives occurs through notifications of a clinical visit to the center regardless of the performance of tests because these are listed as secondary procedures [14]. However, due to the decentralized and outdated nature

Table 1. Diagnostic procedures provided by the Brazilian Policy for Comprehensive Care for People with Rare Diseases

Normative	Main procedure	Secondary procedures
Ordinance No. 199 of January 30, 2014	Assessment for diagnosis of rare diseases – axis I.1 – congenital or late-onset anomalies	Mutation identification by amplicon sequencing up to 500 base pairs (Sanger sequencing) DNA analysis by the Southern blot technique DNA analysis by MLPA Identification of mutations or rearrangements by PCR, methylation sensitive PCR (MS-PCR), qPCR, and methylation sensitive qPCR (MS-qPCR) FISH in metaphase or interphase nucleus Identification of submicroscopic chromosome variation by array-CGH
	Assessment for diagnosis of rare diseases – axis I.2 – intellectual disability	Identification of urinary GAGs) by thin layer chromatography (TLC), electrophoresis, and quantitative measurement Identification of oligosaccharides and sialooligosaccharides by TLC Isoelectric focusing of transferrin (TfIEF) Quantitative dosage of carnitines, acylcarnitine profile Quantitative amino acid assay for diagnosing IEM Quantitative dosage of organic acids for diagnosis of IEM DNA analysis by the Southern blot technique DNA analysis by MLPA Identification of mutations or rearrangements by PCR, MS-PCR, qPCR, and MS-qPCR FISH in metaphase or interphase nucleus Identification of submicroscopic chromosome change by array-CGH Mutation identification by amplicon sequencing up to 500 base pairs
	Assessment for diagnosis of rare diseases – axis I.3 – inborn errors of metabolism (IEM)	Identification of urinary carbohydrates by TLC Identification of urinary GAGs by TLC, electrophoresis, and quantitative measurement Identification of oligosaccharides and sialooligosaccharides by TLC TfIEF Quantitative dosage of carnitines, acylcarnitine profile Quantitative amino acid assay for diagnosing IEM Quantitative dosage of organic acids for diagnosis of IEM Enzymatic assays in plasma, leukocytes, and tissues for diagnosis of IEM Enzymatic assays in erythrocytes for diagnosis of IEM Enzymatic assays in cultured tissue for diagnosis of IEM DNA analysis by MLPA Identification of mutations or rearrangements by PCR, MS-PCR, qPCR, and MS-qPCR
Ordinance No. 1111, of December 3, 2020	Assessment for diagnosis of rare diseases – axis 1.2 – intellectual disability	Whole-exome sequencing (WES)

of this information, access to and monitoring of these financial transfers are impaired. Currently, no structured or published data are available on the number or types of diagnostic tests for RDs performed or accessible through public healthcare services in Brazil.

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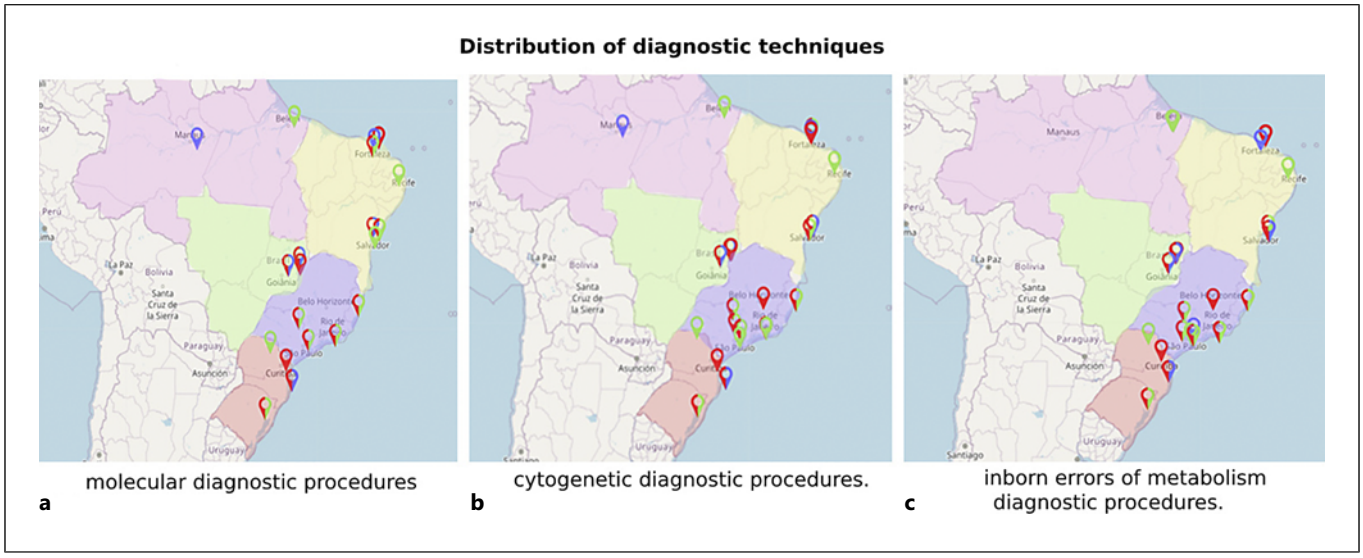

Fig. 1. Distribution of the offered procedures in the Brazilian Network of Rare Diseases (RARAS). Molecular diagnostic procedures (n = 20; **a**), cytogenetic diagnostics (n = 23; **b**), and IEM diagnostic procedures (n = 22; **c**). Regarding the pins, red shows the Reference Services for Rare Diseases (RDRS), blue indicates the Reference Services for Newborn Screening (NSRS), and green represents University Hospitals (UH). On the other hand, the colors in the map represent each of the five regions of Brazil: pink denotes the North, yellow the Northeast, light green the Midwest, purple the Southeast, and light red the South region.

This structured network comprises more than 40 voluntary institutions that offer diagnosis and treatment for RDs in Brazil. It includes 16 UH), four Reference Services for Rare Diseases (RDRS), and four Reference Services for Newborn Screening (NSRS). In addition, other centers of the RARAS Network are allocated to two categories each: eight centers are RDRS and UHs, four centers are RDRS and NSRS, and one center is a NSRS and UH [16, 17]. Based on the data collected in this project, the present study aimed to map the availability and distribute different RD diagnostic procedures cited in the BPCCPRD in RARAS healthcare centers.

Materials and Methods

Study data were collected and managed using Research Electronic Data Capture electronic data capture tools hosted at Ribeirao Preto Medical School, University of São Paulo [18, 19]. A questionnaire was developed based on the diagnostic procedures used to care for people with RDs in the Unified Health System (SUS) according to the BPCCPRD [14] and Ministry of Health Ordinance 1,111 of 2020 [20] (Table 1). To characterize the procedures, the questionnaire presented 22 questions, considering (i) the availability of the procedure in the institution at the healthcare level; (ii) the type of this availability (on-site or outsourced to a public or private service); (iii) the conditions for which the procedure is available, if applicable; and (iv) the average number of procedures performed per month.

Techniques such as karyotyping are not part of the list of tests of the BPCCPRD; nevertheless, karyotyping was investigated in the present study as it was the only procedure covered by the Unified Health System (SUS) prior to the establishment of the policy. Additionally, as next-generation sequencing (NGS) was incorporated into the policy through WES, other NGS techniques such as single- and multi-gene sequencing were researched.

Of the 40 institutions participating in the RARAS Network, 37 center coordinators received an email link to the questionnaire for participation. This is because two centers joined the network after data collection ended and one center was excluded from the analysis due to a lack of data. Data were collected from August 26, 2020 to March 08, 2021 in two steps: (i) sending the questionnaire and collecting data and (ii) verifying the information by contacting those responsible for filling it out to clarify inconsistencies and validating/consolidating the information.

The validated data were exported in csv format and allocated to a shared environment by the researchers to build the analyses. The final stage's objective was to generate reports and graphs based on classic descriptive statistics.

This research study evaluated closed- and open-ended questions. The closed-ended ones were processed and analyzed using the Python programming language and classical statistics, which allowed for the computation of various survey response-descriptive metrics. Combining classical statistical methods with Python programming made it possible to comprehensively analyze the procedures available in RARAS, providing valuable insights into the responses. Open-ended questions were presented in the questionnaire to describe the most common diseases diagnosed by the tests in each center. This project was approved by the Institutional Review Board (IRB) of Hospital de Clínicas de Porto Alegre, the coordinator center (CAAE: 33970820.0.1001.5327), and all the participating institutions' IRBs.

Table 2. Diagnostic procedures available in different centers in the North region

Procedure	North centers		
	Hospital Bettina Ferro de Souza Belém/PA	Policlínica Codajás Manaus/AM	CESUPA Belém/PA
	UH	NSRS	UH
Blood karyotype	Public (20)	Public (3)	
Long-term cultured karyotype with band technique		Public (3)	
Bone marrow karyotype		In site (3)	
Array-CGH	Public (5)		
FISH	Public (5)	Public (2)	
PCR, MS-PCR, qPCR, and MS-qPCR	Public (10)	Public (2)	
Sanger sequencing	Public (3)		
Urinary GAGs	Public (3)		
Plasma and leukocyte enzyme assays for diagnosis of IEM	Public (5)		Private (5)

In site: procedure performed at the center. In parentheses is presented the average number of procedures offered per month by the center.

Results

Distribution of Techniques by Diagnostic Procedure Group

The diagnostic techniques were distributed based on the type of performing center and grouped into three large groups, namely, molecular tests, IEM tests, and cytogenetics as shown in Figure 1. Of the 37 institutions, 27 (73%) provided genetic tests. Of those, 23 (62.16% of the total) offered cytogenetic tests, 20 (54.05%) offered molecular procedures, and 22 (59.46%) offered IEM diagnostic tests.

Figure 1a reveals a shortage in the supply of molecular diagnostic procedures in the North and Midwest regions, where some centers do not offer any tests. The highest availability for this group of procedures was observed in RDRS (in red). The PCR technique was the most available (16/20), followed by multiplex ligation-dependent probe amplification (MLPA) (12/20) and Sanger sequencing (9/20). Only 7 centers reported NGS techniques available – among these, 6 reported availability of WES. Four centers reported having access to the Southern blot technique.

Regarding polymerase chain reaction (PCR) techniques, tests for the following disease diagnoses (Fragile X syndrome, Huntington's disease, Y-chromosome microdeletions, Williams syndrome and methylation for Prader-Willi/Angelman, Silver-Russell and Beckwith-Wiedemann) and genes (*CFTR* and *GALT*) were offered.

Regarding Sanger sequencing, the following disorders and genes were cited: osteogenesis imperfecta (*COL1A1* and *COL1A2* genes), *PTEN*, *MECP2*, *TWIST1*, deafness (*GJB2* and *GJB6*), *FLP3*, *BRAF*, *ITQ*, *TP53*, *BCRABL1*, and the targeted search for familial variants. For NGS, single-gene sequencing and multigene panels were cited (e.g., clinical panels for epilepsies, bone dysplasias, oncogenetics, osteogenesis imperfecta, cystic fibrosis, hereditary spastic paraparesis, developmental delay, and multiple congenital anomalies syndromes).

Regarding the MLPA technique, the centers mentioned the investigation of Duchenne muscular dystrophy, 22q11.1 deletion, Prader-Willi/Angelman syndrome, Rett syndrome, *CYP21A2*, neurofibromatosis type 1, subtelomeric microdeletions, and X-linked intellectual disability.

Considering the distribution of cytogenetic diagnostic procedures presented in Figure 1b, the offers were most highly concentrated in the main cities in the Southeast region of the country. For the fluorescence in situ hybridization (FISH) technique, the diagnosis of Williams syndrome, 5p deletion, 22q11.2 deletion, Prader-Willi/Angelman syndrome, and *SRY* were cited.

In the last group (IEM diagnostic procedures, shown in Fig. 1c), there was a high concentration of procedures in the South and Southeast regions. In the North region, no procedures were offered locally, and only enzyme assays and urinary glycosaminoglycan (GAG) were outsourced. Reference Services for Rare Diseases (RDRS) were also predominant in offering the tests in this group. Considering local and outsourcing availability, of the 37 participating centers, 22 (59.4%) had at least one IEM procedure available. The acylcarnitine profile with or without quantitative carnitine dosage and

Table 3. Diagnostic procedures available in different centers in the Northeast region

Procedure	Northeast centers						
	Hospital Universitário Alcides Carneiro Campina Grande/PB	APAE Salvador/BA	Hospital Universitário Professor Edgar Santos Salvador/BA	Escola Bahiana de Medicina e Saúde Pública Salvador/BA	Hospital Infantil Albert Sabin Fortaleza/CE	Hospital Universitário Walter Cantídio Fortaleza/CE	Hospital Geral Dr. César Cals Fortaleza/CE
	UH	RDRS and NSRS	UH and RDRS	UH	RDRS	UH and NSRS	NSRS
Blood karyotype with band technique	Private (10)	Public (180)	Public (40)		In site (30)	Private (60)	Private (5)
Long-term cultured karyotype with band technique							Private (5)
Bone marrow karyotype			In site (15)		In site (50)	In site (20)	Private (5)
Karyotype (chorionic villus or amniotic fluid)							Private (5)
Array-CGH	Private (10)	Private (4)			Private (2)		
FISH		Private	In site (10)		Private (10)		
Southern blot		Private	Public (10)		Private		
MLPA	Private (5)	Private	Public	Private (4)	Private (2)		
PCR, MS-PCR, qPCR, and MS-qPCR	Private (3)	Private	Public		Private (6)	In site (20)	Private (3)
Sanger sequencing		Private	Public	Private (10)			
NGS (single gene) (panels)	Private (2) (5)	Private		Private (18) (18)		Public (5) (5)	
WES	Private (2)	Private (4)					
TfIEF		Private	Public				
Urinary GAGs	Public (3)	Private	Public				Private
Identification of oligosaccharides and sialooligosaccharides by TLC	Public (2)	Private	Public		Private		Private (2)
Acylcarnitine profile	Public (1)	Private	Public		Private		Private
Quantitative amino acid analysis	Public (2)	Private	Public		Private (5)		Private (2)
Urine organic acids	Public (1)	Private	Public		Private (5)		Private (2)
Plasma and leukocyte enzyme assays for diagnosis of IEM	Public (1)	Private	Public				Private (2)
Erythrocytes enzyme assays for diagnosis of IEM		Private	Public				Private (2)
Enzyme assays on cultured tissue for diagnosis of IEM		Private	Public				
Urinary carbohydrates chromatography	Public (1)	Private	Public				Private (2)

Public/private: procedure outsourced to public/private services. In site: procedure performed at the center. In parentheses is presented the average number of procedures offered per month by the center (when informed). NGS presents both single gene and panels. In parentheses is presented the average number of procedures offered per month by the center (when informed).

Table 4. Diagnostic procedures available in different centers in the Midwest region

Procedure	Midwest centers		
	Hospital de Apoio Brasília/DF[a]	Hospital Materno Infantil Brasília/DF[a]	APAE Anápolis/GO
	RDRS and NSRS	RDRS	RDRS and NSRS
Blood karyotype with band technique	In site (48)	In site (50)	Private (25)
Long-term cultured karyotype with band technique		In site (3)	
Bone marrow karyotype	In site (25)	In site (20)	
Array-CGH			Private (3)
FISH			Private
MLPA		In site (5)	Private (25)
PCR, MS-PCR, qPCR, and MS-qPCR	In site (15)		Private (5)
TfIEF		Public (2)	
Urinary GAGs		Public (3)	Private (10)
Identification of oligosaccharides and sialooligosaccharides by TLC		Public (1)	
Acylcarnitine profile	In site (4,000)	In site (400)	Private (10)
Quantitative amino acid analysis	In site (4,000)	In site (400)	Private (12)
Urine organic acids	Private (10)	Public (10)	Private (12)
Plasma and leukocyte enzyme assays for diagnosis of IEM	Private (20)		Private (10)

[a]Diagnostic tests carried out at the Genetics Unit of Hospital de Apoio de Brasília. Public/private: procedure outsourced to public/private services. In site: procedure performed at the center. In parentheses is presented the average number of procedures offered per month by the center (when informed). NGS presents both single gene and panels procedures monthly average number of exams, shown in the parenthesis.

the quantitative dosage of amino acids were the procedures most frequently performed locally. On the other hand, enzyme assays on cultured tissue were the most frequently outsourced test.

Distribution by Region

To deepen the analysis, this section characterizes each region of the country, presenting the procedures offered, the average number performed per month, and the type of availability in these centers (in-house or outsourced to public and/or private services).

North Region

The North region has the largest territorial area of the five regions of Brazil (over 3 million km^2) and covers 45.25% of the national territory [21]. Most of the Amazon rainforest is in its territory. Despite being the largest region, it is the second least populous in Brazil, with 18.6 million inhabitants and 6.3% of the national gross domestic product (GDP) [22]. Table 2 presents the diagnostic procedures available in the three participating centers in the North region. None of the participating centers in the North region offered prenatal cytogenetic diagnosis or DNA analysis using the Southern blot technique, MLPA, or NGS techniques (single gene, multigene panel, or WES).

Additionally, no center in this demographic region had clinical availability of diagnostic techniques for IEM other than urinary GAG and plasma and leukocyte enzymatic assays. Furthermore, two health centers in the North region, located in Acre State, did not have any procedures available either on-site or accessible via public or private outsourcing.

Northeast Region

The Northeast region has the second largest population (more than 57 million inhabitants) and 14.2% of the national GDP [22] and is the third largest national territory (1.5 million km^2) [21]. Table 3 presents the diagnostic procedures available in the participating centers in the Northeast region. Three centers in the Northeast region participating in the survey did not perform any of the investigated diagnostic techniques.

Midwest Region

The Midwest region has a territorial area of 1.6 million km^2 [21] and is the least populous region in the country, with 16 million inhabitants and 10.4% of the national GDP

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Table 5. Diagnostic procedures available in different centers in the Southeast region

Procedure	Southeast centers								
	UNICAMP Campinas/SP	Instituto da Criança SP/SP	IFF/Fiocruz RJ/RJ	Faculdade de Medicina do ABC Santo André/SP	Hospital São Paulo SP/SP	Hospital Universitário Pedro Ernesto RJ/RJ	Hospital Infantil João Paulo IIBH/MG	Hospital de Clínicas Ribeirão Preto/SP	Instituto Jô Clemente SP/SP
	UH and RDRS	UH	UH and RDRS	UH and RDRS	UH	UH	RDRS	UH and RDRS	NSRS
Blood karyotype with band technique	In site (16)	Public (20)	In site (40)	In site (20)		In site (10)		In site (4)	
Long-term cultured karyotype with band technique			In site (2)	In site (2)		In site (2)		In site (455)	
Bone marrow karyotype		Public		Private (1)		In site (10)		In site (539)	
Karyotype (chorionic villus or amniotic fluid)		Public		In site (1)				Private (539)	
Array-CGH			Public (5)	Private (2)			Private (5)		
FISH			In site (2)						
MLPA			In site (2)	Private (2)				In site (10)	
PCR, MS-PCR, qPCR, and MS-qPCR			In site (2)	Private (2)				Public (7)	
Sanger sequencing			In site (10)	Private (2)				In site (14)	
NGS (single gene) (panels)			In site (24) (24)						
WES			Public (24)	Private (1)					
TfIEF	Public (1)			Private (1)				Private (1)	
Urinary GAGs	Public (1)		Public (2)	Public (1)	In site (20)		Public (5)	Public (2)	
Identification of oligosaccharides and sialooligosaccharides by TLC	Public (1)		Public (2)	Public (1)				Public (2)	
Acylcarnitine profile	Public (4)			Public (1)				Private (3)	In site (2000)
Quantitative amino acid analysis	Public (4)		Public (2)	Public (1)				Private (2)	In site (2000)
Urine organic acids	Public (4)			Public (1)				Private (2)	
Plasma and leukocyte enzyme assays for diagnosis of IEM	Public (2)			Public (1)	In site (10)			Public (1)	
Erythrocytes enzyme assays for diagnosis of IEM	Public (1)			Public (1)	In site (10)			Public (1)	
Enzyme assays on cultured tissue for diagnosis of IEM				Public (1)					
Urinary carbohydrates chromatography				Public (1)				Public (1)	

Public/private: procedure outsourced to public/private services. In site: procedure performed at the center. In parentheses is presented the average number of procedures offered per month by the center (when informed). NGS presents both single gene and panels. Monthly average number of exams is shown in the parenthesis.

Table 6. Diagnostic procedures available in different centers in the South region

Procedure	South centers				
	Hospital de Clínicas Porto Alegre/RS	Hospital Pequeno Príncipe Curitiba/PR	Universidade Estadual de Londrina Londrina/PR	Hospital Infantil Joana de Gusmão Florianópolis/SC	Hospital da Criança Santo Antônio Porto Alegre/RS
	UH and SRDR	RDRS	UH	RDRS and NSRS	UH
Blood karyotype	In site (30)	In site (50)	In site (16)	Private (40)	Private (5)
Long-term cultured karyotype	In site	In site (5)		Private (40)	
Bone marrow karyotype	In site (30)	In site (10)		Private (5)	
Karyotype (chorionic villus or amniotic fluid)	In site (5)				
Array-CGH	In site (10)	Private (20)		Private (18)	
FISH	In site (5)	In site (5)		Private (5)	Public[a] (3)
Southern blot				Private (5)	
MLPA		In site (10)			Public[a] (4)
PCR, MS-PCR, qPCR, and MS-qPCR	In site (30)	Private (10)		Private (5)	
Sanger sequencing		In site (5)	In site (2)		
NGS (single gene and panels)	In site (30) (5)	Private (40) (40)			
WES		In site (3)		Private (2)	
TfIEF	In site (10)	Public (1)		Public (5)	Private (1)
Urinary GAGs	In site (20)	Public (3)	Private (5)	Public (5)	Private (1)
Identification of oligosaccharides and sialooligosaccharides by TLC	In site (10)	Public (2)		Public (5)	Private (1)
Acylcarnitine profile	In site (15)	Private (5)		Public (5)	Private (1)
Quantitative amino acid analysis	In site (30)	Private (5)		Public (5)	Private (1)
Urine organic acids	In site (30)	Private (5)		Public (5)	Private (1)
Plasma and leukocyte enzyme assays for diagnosis of IEM	In site (30)	Private (1)		Public (5)	Private (1)
Erythrocytes enzyme assays for diagnosis of IEM	In site (30)	Private (1)		Public (5)	Private (1)
Enzyme assays on cultured tissue for diagnosis of IEM				Public (5)	Private (1)
Urinary carbohydrates chromatography	In site (10)	Private (1)		Public (5)	Private (1)

[a]Performed at Universidade Federal de Ciências da Saúde de Porto Alegre. Public/private: procedure outsourced to public/private services. In site: procedure performed at the center. NGS presents both single gene and panel procedures monthly average number of exams, shown in the parenthesis. In parentheses presented the average number of procedures offered per month by the center (when informed).

[22]. Table 4 presents the diagnostic procedures available in the participating centers in the Midwest region. None of the participating centers in the Midwest region performed prenatal karyotyping techniques or DNA analysis by Southern blotting, Sanger sequencing, or NGS. Additionally, no center in this region offered enzymatic assays in erythrocytes or cultured tissue to diagnose IEM or chromatography of urinary carbohydrates. Three centers in the Midwest region participating in the survey did not perform any of the diagnostic techniques researched.

Southeast Region

The Southeast region of Brazil is the second smallest region in the country, with a territorial area of 924,000 km^2 [21]. It is the most developed and populated, with more than 89 million inhabitants and 55.2% of the national GDP [22]. Table 5 presents the diagnostic procedures available in the 9 participating centers in the Southeast region. Two centers in this region participating in the survey did not perform any of the researched techniques.

Table 7. Monthly average of procedures offered by RARAS Network

Procedure	BPCCPRD axes	Regions of Brazil					Total RARAS Network	Total
		North	Northeast	Midwest	Southeast	South		Ministry of Health of Brazil* (DATASUS)
Blood karyotype with band technique	–	23	325	123	110	141	749	1,183
Long-term cultured karyotype with band technique	–	3	5	3	461	45	517	201
Bone marrow karyotype	–	3	90	45	550	45	733	569
Karyotype (chorionic villus or amniotic fluid)	–	0	5	0	540	5	550	569
Array-CGH	I.1, I.2	5	16	3	12	48	84	5
FISH	I.1, I.2	7	20	0	2	18	47	0
Southern blot	I.1, I.2	0	10	0	0	5	15	0
MLPA	I.1, I.2, I.3	0	11	30	14	14	69	0
PCR, MS-PCR, qPCR, and MS-qPCR	I.1, I.2, I.3	12	32	20	11	45	120	3
Sanger sequencing	I.1, I.2	3	10	0	26	7	46	0
NGS (single gene)	–	0	25	0	24	70	119	0
NGS (panels)	–	0	28	0	24	45	97	0
WES	I.2	0	6	0	25	5	36	0
TfIEF	I.2, I.3	0	0	2	3	17	22	0
Urinary GAGs	I.2, I.3	3	3	13	31	34	84	0
Identification of oligosaccharides and sialooligosaccharides by TLC	I.2, I.3	0	4	1	6	18	29	0
Acylcarnitine profile	I.2, I.3	0	1	4,410	2008	26	6,445	3
Quantitative amino acid analysis	I.2, I.3	0	9	4,412	2009	41	6,471	5
Urine organic acids	I.2, I.3	0	8	32	7	41	88	2
Plasma and leukocyte enzyme assays for diagnosis of IEM	I.3	10	3	30	14	37	94	0
Erythrocytes enzyme assays for diagnosis of IEM	I.3	0	2	0	13	37	52	0
Enzyme assays on cultured tissue for diagnosis of IEM	I.3	0	0	0	1	6	7	0
Urinary carbohydrates chromatography	I.3	0	3	0	2	17	22	88

*Monthly average calculated based on 5 years (2015–2019) [23].

South Region

The South region of Brazil is the smallest of the five regions of the country, with a territorial area of 576,000 km^2 [21], a population of 30 million inhabitants, and 17.2% of the national GDP [22]. Table 6 presents the diagnostic procedures available at the five participating centers in the South region. All centers in the South region had at least one surveyed exam available.

In general, Southern blot analysis, enzyme assays on cultured tissue and urinary organic acid tests measurement had the highest outsourcing rates. On the other hand, the procedures most frequently performed on-site were bone marrow karyotype and long-term cultured karyotype with band technique.

Additionally, ten national centers (UH: 7; NSRS: 1; NSRS+UH: 1; RDRS+UH: 1) without any of the techniques available on-site or outsourced were identified. In addition to the analysis of the distribution of the procedures offered by the centers, it was possible to assess the average number of procedures performed. Table 7 presents the total numbers collected for Brazil and by region. The last column contains data gathered from online public information from the Ministry of Health of Brazil database, the Department of Computer Science of the SUS (Departamento de Informática do Sistema Único de Saúde, DATASUS) [23], to compare our data with official federal notifications. A discrepancy was observed between the data provided by the participating services and the data from

the Ministry of Health. The data provided by the centers in this study tended to show higher production numbers than reported by DATASUS. There was no record of cytogenetic techniques such as FISH; molecular tests such as Southern blot, MLPA, Sanger sequencing, and NGS; and metabolic tests such as isoelectric focusing of transferrin (TfIEF), urinary GAG, oligosaccharides, and sialooligosaccharides by thin-layer chromatography, plasma, and enzyme assays on cultured tissue, leukocytes, and erythrocytes between 2015 and 2019 according to DATASUS. However, according to our data, they were effectively carried out in some centers.

Discussion

Our data showed large discrepancies in the availability of tests in the North and Midwest regions compared to other regions of Brazil, with a greater concentration in the South and Southeast regions, as previously reported in the literature [7, 11]. The high regional discrepancy in this supply can hamper and delay the diagnosis of RDs.

Despite the BRCCPRDs great advancement of incorporating several biochemical and molecular tests into the SUS, there is currently an absence of diagnostic procedures for axis 2 conditions (rare non-genetic diseases) [14]. It is estimated that up to 20% of RDs do not have genetic causes and fall outside the scope of diagnostic procedures under the rare diseases policy [4]. This may result in the potential underdiagnosis and subsequent undertreatment of these rare conditions.

Before the implementation of the BPCCPRD, data from Brazilian studies showed that diagnostic genetic tests were available in 47 (71%) of the 66 genetic services listed in the public health system, partially funded by the SUS. Of these, 83% offered conventional cytogenetics, 55% had high-resolution cytogenetics, 32% had FISH, 36% had IEM tests, and 32% had prenatal diagnosis. About 50% also carried out research using molecular biology techniques [7, 11]. In these previous studies, only medical genetics services were evaluated, and the availability of specific molecular diagnostic techniques was not researched.

Although the questionnaires did not cover the same institutions evaluated in the previous studies, it is crucial to note that the availability of tests was around 70% in both investigations (73% in the RARAS Network); however, the distribution by technique showed discrepancies [7, 11]. For instance, IEM techniques and molecular biology were more frequently offered in the RARAS Network, while prenatal diagnosis, cytogenetic exams, and FISH had greater availability in the previous studies [7, 11]. It should also be noted that the present study included neonatal screening centers, which do not usually carry out karyotyping in their care routine; this could explain its lower rate.

The present survey was carried out with information only on tests available at the clinical assistance level and did not include tests performed through clinical or experimental research in these centers. It is possible, therefore, that other tests may be available at the participating services. However, the tests offered through research can be seasonal and do not involve a continuous flow of financial transfers to the centers. It is known, however, that it is difficult to separate the objectives of research and public health [9].

A discrepancy was observed between the data reported by the services in this research and the numbers released by the Ministry of Health. The data analysis of the DATASUS suggests underreporting and that these techniques were possibly not registered despite being performed by the centers [23]. When procedures are not registered in official databases, it can be challenging to build precise public policies. It also potentially reduces formal investments in technological and human resources for centers that perform such techniques.

In some centers in the Midwest and Southeast regions, a large discrepancy was observed in the number of procedures performed, especially for acylcarnitine profile and amino acids. This may be due to the availability of expanded newborn screening in NSRS in São Paulo and Brasília [24, 25], which is not universal across Brazil. Therefore, such techniques may be passed on through specific payments for newborn screening and are not computed in DATASUS as specific procedures. It is estimated that expanded neonatal screening will be offered in all Brazilian states from 2023 [26].

Ten participating centers did not provide any of the diagnostic tests. Two of these centers are in the North region, three in the Northeast region, three in the Midwest region, and two in the Southwest region. It is possible that the unavailability of such diagnostic tests could necessitate referrals for evaluation and investigation at other centers, which could delay diagnosis and treatment. Future studies are needed to understand whether the unavailability of these diagnostic methods impacts the age of diagnosis and/or diagnostic capability.

It is also worth mentioning that misinformation may have occurred in filling out the form: there are at least two NSRS that reported the unavailability of all the researched techniques. However, they perform neonatal screening, which includes at least two of the investigated procedures (analysis of biotinidase enzyme activity and quantitative measurement of the amino acid phenylalanine).

In this study, the least frequently outsourced tests, performed on-site in most centers, were cytogenetic techniques: long-term cultured karyotyping and bone marrow karyotyping. On the other hand, the most frequently outsourced procedures were Southern blot analysis, enzyme assays on cultured tissue for the diagnosis of IEM, and urinary organic acid tests. Outsourcing of techniques was addressed to both public and private services. As many areas of the country do not have adequate medical genetics support, some of the outsourcing to public services is carried out through diagnostic support networks designed to expand access to information and facilitate the diagnosis of rare genetic diseases. Cases in point include Inborn Errors of Metabolism Network Brazil (*Rede EIM*), Lysosomal Storage Disorders Brazil Network (*Rede DLD*), MPS-Mucopolysaccharidosis Brazil Network (*Rede MPS Brasil*), Niemann-Pick C-NPC Brazil Network (*Rede NPC Brasil*), and MSUD-Maple Syrup Urine Disease Network (*Rede DXB*) [10].

Currently, specific treatments are available for some RDs, such as IEM, including enzyme replacement, substrate reduction, diet therapy, and/or supplementation of enzyme cofactors, increasing life expectancy and quality of life and reducing mortality in many individuals. Therefore, early diagnosis is essential to minimize the struggles experienced by subjects with RDs and their families [2].

Although traditionally considered highly complex and costly procedures, genetic tests are a key step for diagnosing most genetic diseases, evaluating at-risk family members, and performing accurate genetic counseling [13]. The results of this research are expected to support the construction and improvement of public health policies in Brazil.

This multicenter study by RARAS revealed the discrepancy in the availability of diagnostic procedures in the Brazilian territory and the mismatch between the procedures performed and the data from official databases. Wide collaboration between services was identified, with the detection of the outsourcing of multiple diagnostic techniques.

Further studies of the RARAS Network are warranted to confirm the relationship between the availability of these techniques and the rates of RD diagnosis in medical centers. Moreover, tracking the progress of BPCCPRD implementation in Brazil and monitoring the growth of the RARAS Network are also crucial points of investigation.

Collaboration between services that assist individuals with rare diseases should be stimulated. Additionally, specialized laboratories for genetic analysis with expertise and available funding should be established in the country. This work confirms the importance of mapping the services that diagnose and treat individuals with RDs to provide better assistance to such individuals.

Statement of Ethics

This study protocol was reviewed and approved by the Institutional Ethics Committee Board (IRB) of Hospital de Clínicas de Porto Alegre (approval number: 33970820.0.1001.5327), the coordinator center for the study, and in all participating centers – Fundação Universidade Federal do Mato Grosso do Sul (33970820.0.3096.0021); Instituto Jô Clemente (33970820.0.3069.8647); Fundação do ABC – FMABC (33970820.0.3064.0082); Hospital Universitário Júlio Muller-MT (33970820.0.3085.5541); Fundação Hospitalar do Estado de Minas Gerais (33970820.0.3022.5119); Hospital Universitário Walter Cantídio da Universidade Federal do Ceará (33970820.0.3025.5045); Universidade Estadual de Londrina (33970820.0.3034.5231); Hospital das Clínicas do Acre (33970820.0.3016.5009); Escola Bahiana de Medicina e Saúde Publica (33970820.0.3084.5544); Policlínica CODAJAS - Fundação de Hematologia e Hemoterapia do Amazonas (33970820.0.3076.0009); Universidade Evangélica de Goiás (33970820.0.3081.5076); Hospital da Criança Santo Antônio - Santa Casa (33970820.0.3045.5683); Hospital Universitário Pedro Ernesto (33970820.0.3035.5259); Hospital de Clínicas da UNICAMP (33970820.0.3014.5404); Irmandade Santa Casa de Vitória (33970820.0.3020.5065); Hospital Infantil Albert Sabin (33970820.0.3004.5042); Universidade Federal de São Paulo (33970820.0.3028.5505); Hospital Universitário Lauro Wanderley (33970820.0.3029.5183); Hospital Geral Dr. César Cals (3397 0820.0.3066.5041); Maternidade Climério de Oliveira (33970 820.0.3032.5543); Hospital das Clínicas da Faculdade de Medicina de Ribeirão Preto da USP (33970820.0.3010.5440); Hospital Universitário João de Barros Barreto (33970820.0.3062.0017); Hospital Materno Infantil de Brasília (33970820.0.3046.5553); Instituto da Criança do HCFMUSP (33970820.0.3021.0068); Hospital Universitário Prof. Edgard Santos da Universidade Federal da Bahia - HUPES/UFBA (33970820.0.3001.0049); Escola Bahiana de Medicina e Saúde Publica (33970820.0.3042.5544); Hospital de Apoio de Brasília (33970820.0.3046.5553); Centro Universitário do Pará (33970820.0.3052.5169); Hospital de Crianças César Pernetta e Hospital Pequeno Príncipe (33970820.0.3002.0097); Hospital Infantil Joana de Gusmão (33970820.0.3009.5361); Hospital Universitário Clementino Fraga Filho da Universidade Federal do Rio de Janeiro (33970820.0.3079.5257); Instituto de Psiquiatria do Hospital das Clínicas de São Paulo (33970820.0.3040.0068); Hospital Universitário Alcides Carneiro da Universidade Federal de Campina Grande (33970820.0.3006.5182); and Instituto Fernandes Figueira - IFF/FIOCRUZ (33970820.0.3007.5269). Written informed consent was not obtained as the involvement in this project extended beyond individual participants to institutions, and this requirement was waived by the respective IRBs.

Conflict of Interest Statement

None of the authors has a financial or other conflict of interest.

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Author Contributions

B.M.O., M.B.N., and T.M.F. participated in the construction of the REDCap form. M.B.N. and I.C. participated in data collection. B.M.O., M.B.N., and I.C. participated in the data quality review. T.M.F. and D.A. participated in the study design. B.M.O., M.B.N., I.C., and D.A. participated in the data analysis. B.M.O., M.B.N., and I.C. drafted the manuscript. T.M.F., I.V.D.S., and D.A. contributed with manuscript revisions. All have given final permission to submit for publication.

Data Availability Statement

All data analyzed in this study are available in detail through the RARAS online portal: https://raras.org.br/index.php/procedimentos/ (https://doi.org/10.25504/FAIRsharing.d7b6c8). For any further inquiries, please feel free to contact the corresponding author.

Appendix

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