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ORIGINAL ARTICLE

Clinical and Genetic Findings from a Rare Pediatric Diseases Outpatient Clinic in Curitiba, Brazil

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Keywords:
Rare Diseases, Genetic profile, Clinical diagnosis, Whole Exome Sequencing Polymorphism, Single Nucleotide Karyotype.

Abstract

Introduction: Rare diseases are chronic, progressive conditions with a low frequency of occurrence in the population. They can be divided into two groups: diseases of genetic origin and of non-genetic origin. Currently, molecular genetic tests are widely used to screen and diagnose patients with rare diseases. This study aims to describe the genetic/clinical profile of patients seen at the Rare Diseases Outpatient Clinic of a referral pediatric hospital in southern Brazil. **Methodology:** This retrospective descriptive quantitative study looked into data from paper and electronic medical charts of patients seen at the Rare Diseases Outpatient Clinic and genetic test results of included patients stored in the platform of the hospital's Clinical Analysis Laboratory. **Results and Discussion:** The study included 553 patient medical charts. Prevalence of male patients and aged between zero and 11 years was greater. The most commonly reported symptoms were dysmorphisms and congenital malformations (12.5%), developmental delay (11.0%) and seizures (10.5%). Only 24.7% of the molecular genetic tests presented altered results. Alterations were more notably seen in genes PHKA2, G6PC, FBP1, and CTNS. The number of altered test results was lower than expected. The greater availability and popularization of these tests can be noticed by the increase in the number of tests ordered. Increased awareness about molecular genetic testing is very positive and may help healthcare teams improve patient quality of life.

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INTRODUCTION

Rare diseases are conditions with a low frequency of occurrence in the population. They affect affecting up to 1.3 people per 2,000 population[1]. It has been estimated that there are between 6,000 and 8,000 rare diseases, whose symptoms are different and vary not only among different diseases but also among different patients, which makes diagnosis difficult[2,3].

In general, rare diseases are chronic, progressive, disabling, incurable conditions. Multidisciplinary follow-up is necessary for treatment and/or prevention of symptoms. These diseases are divided into two groups according to the etiology of alterations: conditions of genetic origin, which account for approximately 80% of all rare disease, and conditions with non-genetic causes, related to inflammation, infection, autoimmunity, allergy, and environmental causes[2,3].

Since this is a heterogeneous group of disorders, the diagnosis of rare diseases is still considered a challenge, and is often performed years after the onset of symptoms. Moreover, due to the low prevalence, there is little epidemiological and clinical data available, and there are no well-established criteria for the diagnosis of some rare diseases and/or a lack of knowledge of healthcare teams to guide diagnostic investigations[4,5,6].

Early diagnosis is essential, since most rare diseases may cause severe symptoms if not treated appropriately. Currently, in addition to biochemical tests, molecular genetic tests can be used to identify alterations related to certain diseases even before the onset of symptoms. Molecular genetic tests also provide input for genetic counseling, so that the families of patients with rare diseases are informed about the probability of recurrence of the condition and to find and diagnose early on other individuals who may develop it[3].

Despite having low prevalence in the population, rare diseases must be the object of attention of healthcare teams. Considering the scarcity of data in the literature and the relevance of genetic tests in confirming diagnosis, it is fundamental to learn the profile of patients seen in our service, since such knowledge may improve our clinical and laboratory teams to find and treat this population more efficiently.

This study aims to describe the genetic-clinical profile of the patients seen at the Rare Diseases Outpatient Clinic of a referral pediatric hospital in southern Brazil. More specifically, it describes how often tests with genetic alterations occur in our patient population, lists the most common alterations, and relates genetic alterations to patient clinical symptoms.

METHOD

This is retrospective descriptive quantitative study was carried out in a referral pediatric hospital in the city of Curitiba, PR, Brazil, accredited as a Referral Service in Rare Diseases since 2016.

Data collection was initiated after approval was given by the institution's Research Ethics Committee (certificate 32145020.6.0000.5580). The medical charts of patients seen at the Rare Diseases Outpatient Clinic and genetic test results available on the interface platform connecting the Clinical Analysis Laboratory and the Hospital were used as sources of information.

Participants were included considering the pre-established inclusion and exclusion criteria. Patients seen at the outpatient clinic between September 2016 and July 2020 with molecular genetic tests with specimens collected at the hospital were included in the study. Patients seen before September 2016 and as of August 2020, patients who did not have specimens collected for molecular genetic testing at the hospital, and patients whose medical charts were incomplete and/or did not clearly describe symptoms were excluded from the study.

The collected data were processed on Microsoft Excel, analyzed, and presented based on exploratory descriptive statistical analysis and inferential statistical analysis using the chi-squared test.

RESULTS

We included 553 patient charts, 324 (58.6%) belonging to males and 229 (41.4%) to females. For purposes of describing age groups, we considered the age of each patient at their last visit. Patient mean age was 6.26 years (±4.81). The youngest patient was three months old and the oldest was aged 22 years. Patients were split into age groups, as provided in the Child and Adolescent Statute[7]. The majority of patients – 458 (82.8%) – were children aged 0-11 years; 83 (15%) were adolescents; and 12 (2.2%) were adults.

Included patients suffered from a wide range of symptoms (Figure 1). The most frequently observed symptoms were dysmorphisms and congenital malformations (12.5%), followed by global development delay (11.0%), and seizures (10.5%). Other symptoms such as hypotonia (5.2%), aggressiveness (3.8%), intellectual disability (3.2%), microcephaly (3.5%), and vision alterations (3.1%) were also identified in large portion of the patients. Key: GDD – global developmental delay; GDR – global developmental regression.

Regarding parental consanguinity, we found 26 medical charts (4.7%) that reported the presence of family consanguinity and 243 (43.9%) that reported no consanguinity. More than half (51.4%) of the analyzed medical charts did not contain this information and were therefore considered as cases in which there was no parental consanguinity.

We found 691 molecular genetic tests performed in our patient population. Of these, 520 (75.3%) had unaltered results and 171 (24.7%) had anomalous results. The tests were categorized by type in order to identify which had been the most frequently ordered during the study period. Table 1 shows the frequencies of each type of ordered test. The most frequently performed tests were karyotyping (33.9%), screening for chromosomal abnormalities by Single Nucleotide Polymorphism array (SNP array; 22.7%) and molecular newborn screening (16.5%).

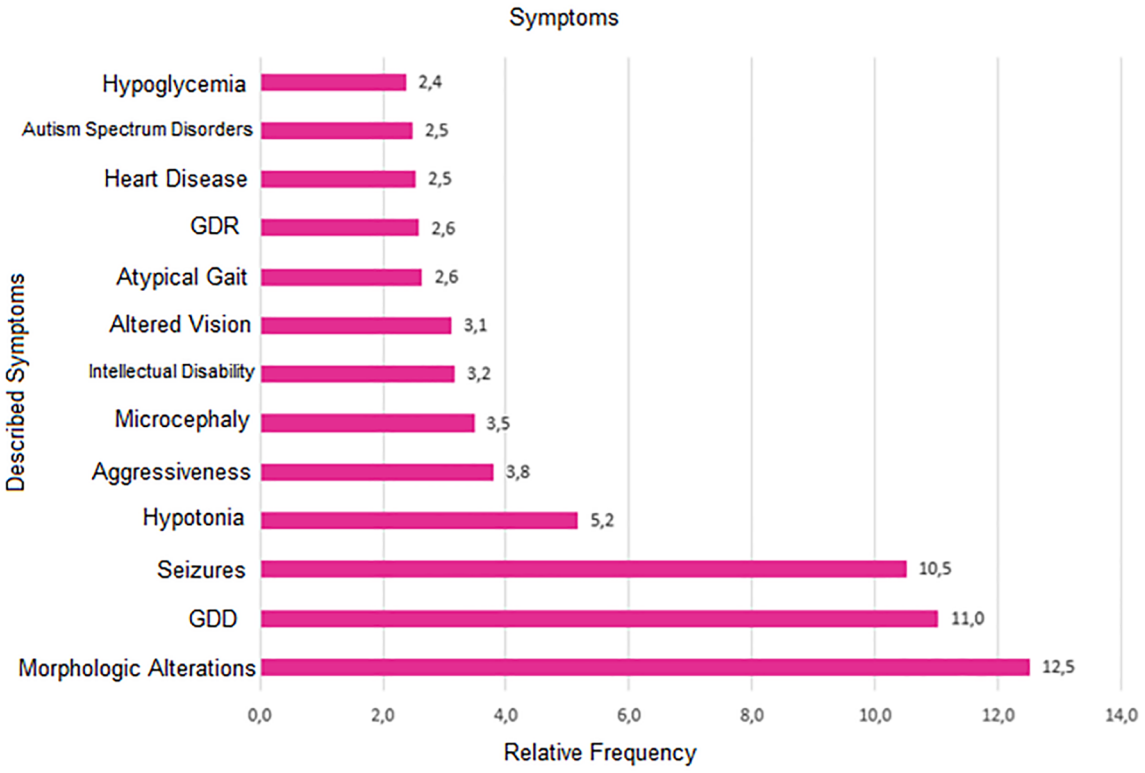

Figure 1. Symptoms described in the patient population [Symptoms | Described Symptoms – Hypoglycemia; Autism Spectrum Disorders; Heart Disease; GDR; Atypical Gait; Altered Vision; Intellectual Disability; Microcephaly; Aggressiveness; Hypotonia; Seizures; GDD; Morphologic Alterations | Relative Frequency] Key: GDD – global developmental delay; GDR – global developmental regression.

Table 1. Frequency of each type of ordered test

Tests	Absolute Frequency (n)	Relative Frequency (%)
Karyotyping	234	33.9
SNP Array	157	22.7
Molecular newborn screening	114	16.5
Alterations in a single gene/region	45	6.5
Treatable diseases panel	42	6.1
Exome	41	5.9
Epilepsy panel	40	5.8
Other panels	14	2.0
Inborn errors of metabolism panel	4	0.6
Total	691	100

The graph in Figure 2 shows the proportions of tests with and without alterations by test type. More than half (53.7%) of the exome sequencing and 50% of the gene sequencing tests designed to find inborn errors of metabolism yielded anomalous results. In the other test types, the number of tests without alterations was greater than the number of tests with altered results.

We also looked into the numbers of tests ordered over the years in the outpatient clinic. Figure 3 shows the absolute number of tests order annually. We found records of tests performed in 2016. The mean number of tests ordered in 2017 was 0.8 per month, while 2019 had the highest average on record, with 31.7 tests prescribed and performed per month. In 2020, the mean number of tests dropped to 13.4 per month.

Most of the genetic alterations found in the tests were genetic (52.0%). Chromosomal alterations accounted for 43.3% of the total. Epigenetic (2.9%) and mitochondrial (1.8%) alterations were also described.

Genetic alterations were identified in 79 different genes, with genes PHKA2, G6PC, FBP1 and CTNS ranking on the top of the list. Chromosomal alterations were found in all chromosomes, with the exception of chromosome 5. Chromosomes 17 and 18 presented the highest frequency of alterations.

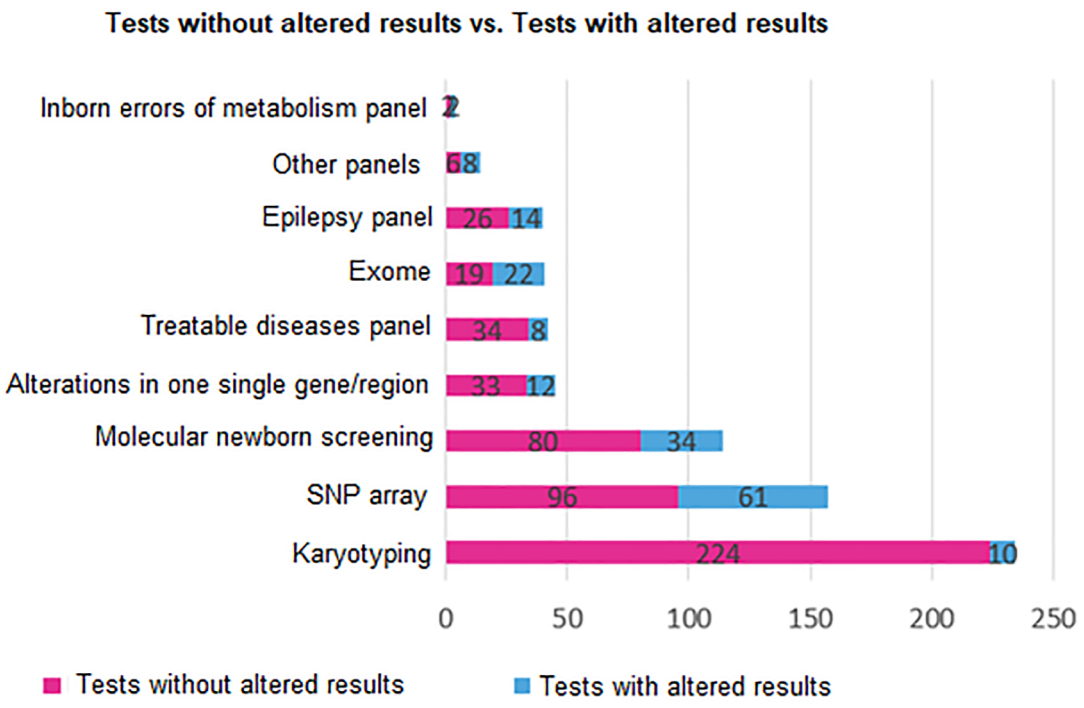

Figure 2. Ratios between tests with and without altered results by test type. [Tests without altered results vs. Tests with altered results | Inborn errors of metabolism panel; Other panels; Epilepsy panel; Exome; Treatable diseases panel; Alterations in one single gene/region; Molecular newborn screening; SNP array; Karyotyping | Tests without altered results; Tests with altered results].

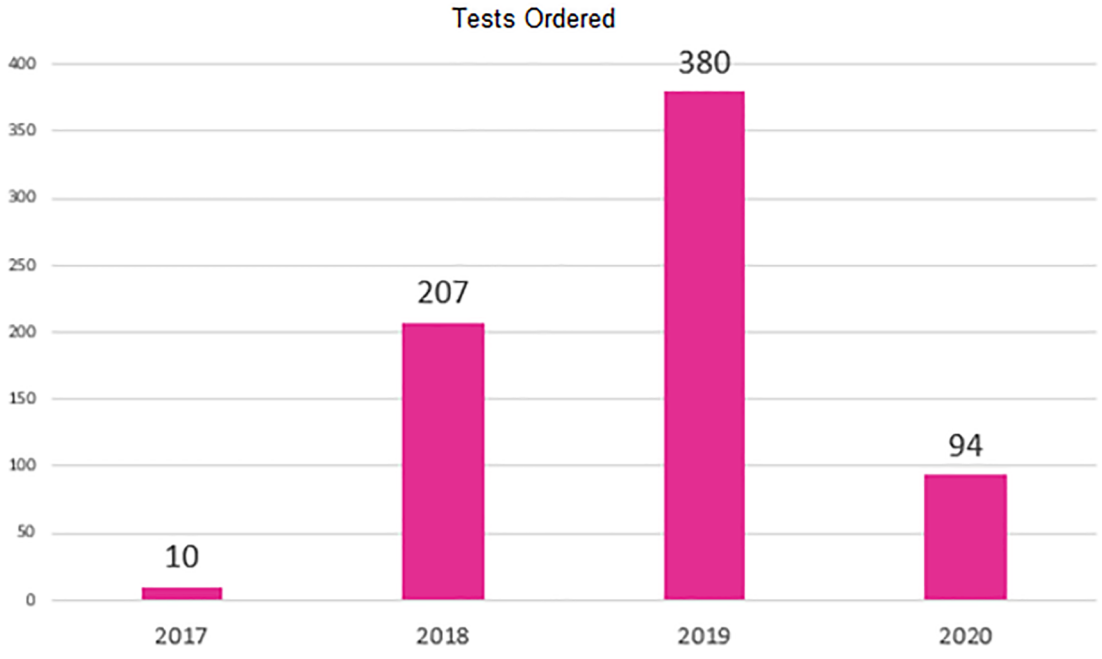

Figure 3. Total number of tests ordered per year.

The chi-squared test was used to assess the relationship between some variables and the occurrence of anomalous test results with confidence level of 95%. Thus, if a p-value of less than 0.05 is obtained, the null hypothesis is rejected and, therefore, the existence of a relationship between the variables analyzed is suggested.

The analysis of potential associations between gender and presence of altered test results and presence of the most frequent symptoms and tests with altered results yielded p-values of 0.22 and 0.98, respectively. This finding suggests that there is no association between gender or the presence of the most frequent symptoms and the occurrence of altered test results.

The evaluation of a potential association between consanguinity and presence of altered test results found a statistically significant difference between the groups (p=0.001). Thus, it is possible to state that the presence of parental consanguinity is associated with a higher probability of test results with genetic alterations.

DISCUSSION

Considering that early diagnosis is paramount for the quality of life of patients with rare diseases, the fact that the population seen at the outpatient clinic in question is mostly comprised of children is of great relevance. Analyses of the age range of the included patients showed that 58.6% of them were in early childhood, with ages between 0 and 6 years. This indicates that patients have been sent to a referral center at an early age, shortening the path they usually go through during the investigation of their cases.

A wide range of non-specific symptoms were described in our patient population, as suggested in the literature[8,9]. The most frequently described symptoms were common to several diseases, rare and not, of genetic origin and with other etiologies. This is the main factor responsible for incorrect diagnosis and delays in the initiation of treatment, since care teams are often unaware of the symptoms associated with rare diseases[10,11,12].

A remarkable finding was the substantial number of medical charts without information about parental consanguinity (n=284), given the relevance of this information in diagnostic investigation. Even the absence of parental consanguinity should be mentioned in medical charts for future reference. Parental consanguinity has been associated with genetic disorders and infant mortality in different populations[13,14].

In the study population, the relationship between consanguinity and presence of genetic alterations indicated a greater likelihood of children born from consanguineous couples having anomalous genetic test results. Knowledge of family history and existence of parental consanguinity is important not only to search for possible parental genetic and phenotypic alterations, but also to advise families of the possibility of other children having the same manifestations.

With the development of molecular techniques, molecular genetic tests have been used in the diagnosis of different diseases, although they are not widely available. Karyotyping is among the simplest and fastest of tests. Thus, although it does not allow the detection of deletions or duplications smaller than 5 Mb, this test is more accessible and may be enough to diagnose or guide the diagnostic investigation of some rare diseases, working as an initial screening for chromosomal abnormalities[15]. This aspect alone explains why this is the most ordered test for outpatients.

According to Miller et al. (2010)[16] and the recommendations of the American College of Medical Genetics and Genomics for the use of microarray techniques in clinical practice[17], the SNP array test, also used to identify chromosomal anomalies, is the most indicated test in the initial assessment of individuals with symptoms such as congenital malformations, dysmorphisms, global developmental delay, and intellectual disability. These are among the most frequent symptoms (26.7%) seen in our study population, which explains why this test has been ordered so often in our service.

The development of Next Generation Sequencing (NGS) and the increased knowledge about gene function have made testing for mutations possible. Sequencing scans may look for changes in a single gene, a specific set of genes or the entire exome[18]. The determination of which genes are covered in sequencing depends on diagnostic suspicion and medical prescription.

Newborn genetic screening performed from specimens collected with a buccal swab is one of the best-known tests. The test was developed by a Brazilian laboratory and offers a genetic panel that allows the identification of gene alterations related to more than 300 treatable diseases with symptoms that may manifest in early childhood[19].

The proportion of anomalous test results in the study population was lower than expected, considering that 80% of rare diseases are genetic[3]. However, this may be due to the fact that not all patients had a definite diagnosis and therefore were still having their symptoms investigated. New tests may be performed in the future to identify genetic alterations in the patients who did not have altered test results in this study.

Exome tests allow the assessment of the whole coding DNA through sequencing. Thus, alterations in a large number of genes can be identified and analyzed based on the knowledge available about gene function[18]. Exome tests have assisted in the investigation of patients with possible rare diseases and greatly accelerated diagnosis, allowing treatment to begin earlier[20]. In our study, we noticed that exome tests were the ones with the highest proportion of altered results. This may be related to the large number of genes studied, allowing the identification of alterations in genes not covered by other genetic panels.

However, it is important to emphasize that, even though they are more likely to identify genetic alterations in patients, exome tests are very expensive and often not covered by health insurance. Thus, health care teams that prescribe tests tend to order less expensive tests to initiate patient investigations in an attempt to obtain conclusive results from screening. This also explains why karyotyping, with only 4.3% of altered results in our study, is the most ordered screening test.

We found that not all genes with the highest frequency of alterations in the study population have their function well described in the literature. The symptoms experienced by the study population with alterations in these genes are in line with the symptoms described in the literature.

The PHKA2 gene is responsible for encoding the alpha subunit of liver enzyme phosphorylase kinase b, an enzyme of fundamental importance in glycogen breakdown[21]. The symptoms derived from alterations in this gene include hepatomegaly, failure to thrive, hypoglycemia, hyperlactatemia, and ketonuria[22,23], all of which described in our patient population with alterations in this gene.

The G6PC gene encodes enzyme glucose-6-phosphatase (G6PC), involved in glycogenolysis and gluconeogenesis. Mutations in this gene may trigger hepatomegaly, hypoglycemia, increased uric acid and triglyceride levels, and lactic acidosis[24]. Hepatomegaly and hypoglycemia were the main symptoms described for patients with alterations in gene G6PC in our study (n=5).

Pathogenic changes in the FBP1 gene can cause deficiency or loss of function of enzyme fructose 1,6-biphosphatase (FBPase), which helps regulate gluconeogenesis. The symptoms most often seen in patients with these alterations are acute bouts of lactic acidosis and ketotic hypoglycemia[25]. All patients (n=4) with FBP1 gene alterations had hypoglycemia as the main symptom.

Alterations in the CTNS gene trigger cystinosis, in which cystinosin accumulation can lead to kidney dysfunction, polyuria and polydipsia, dehydration, metabolic and electrolyte alterations, and hypothyroidism[26,27]. In our study, patients with mutations in this gene had metabolic acidosis, hypertension, hypothyroidism, and kidney dysfunction.

Interestingly, the genes more frequently associated with altered test results in this study relate to inborn errors of metabolism, the most common type of genetic rare disease in our outpatient clinic.

With regard to chromosomal alterations, even though some chromosomes had more alterations than others, the regions in which such alterations occurred in the chromosomes varied. Each chromosome carries a large number of genes that encode proteins involved in different processes and tissues. Thus, for each chromosomal alteration, there are distinct clinical manifestations that may be used to characterize certain diseases.

Variants of uncertain significance (VUS) were present in the performed tests. Variants in this category have poorly defined or contradictory criteria for defining pathogenicity or benignity in the literature[28].

Due to technical progress and the findings of population studies, it is possible that variants once categorized as of uncertain significance lose this status, which can help in patient clinical evaluation, diagnosis, and treatment. Thus, it is also important that laboratories in which genetic tests are performed and healthcare teams involved in the care of these patients advise them to retest periodically, so that they always have access to current information about their diseases[28].

We noticed a significant increase in the number of tests ordered since the consolidation of the outpatient clinic. In 2018, the total number of tests ordered (n=207) was 20.7 times greater than the number seen the previous year (n=10). In 2019, the increase was 1.8 times relative to 2018. This increase is likely related to the greater availability and popularization of these tests among healthcare workers and patient families. The drop in the number of tests in 2020 was caused by the COVID-19 pandemic. Preventive measures to curb the spread of COVID-19 caused a substantial decrease in the number of face-to-face consultations and, consequently, on the number of tests ordered.

Molecular genetic tests play a key role in the diagnosis of rare diseases of genetic etiology. In our study, the number of anomalous test results was lower than expected, but its relevance in differential diagnosis is clear. The greater availability of these tests is clearly reflected in the sizeable increase in the number of specimens collected at our outpatient clinic.

We found a higher prevalence of gene alterations linked to inborn errors of metabolism in our population.

Early referral to specialized centers is a positive indication of the greater level of awareness of healthcare teams and a key element in improving patient quality of life.

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