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ORIGINAL ARTICLE

Next generation sequencing in pediatric bone marrow failure: a valuable tool for accurate diagnosis

Secuenciación de nueva generación en falla medular pediátrica: una herramienta valiosa para un diagnóstico preciso

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What do we know about the subject matter of this study?

Inherited bone marrow failure syndromes include a heterogeneous group of etiologies and account for approximately 25% of cases of bone marrow failure in the pediatric population. Next-generation sequencing technologies have emerged as an option for accurate etiologic diagnosis in this group of patients.

What does this study contribute to what is already known?

This study provides evidence for the usefulness of next-generation sequencing studies for the etiologic diagnosis of bone marrow failure in pediatric patients in countries with emerging economies. In addition, we describe two new variants in the FANCA and PARN genes.

Abstract

Inherited Bone Marrow Failure syndromes account for approximately 25% of cases of aplastic anemia in pediatric patients. Next-generation sequencing (NGS) technologies have allowed the diagnosis of an increasing number of hereditary causes of bone marrow failure. **Objective:** To determine the diagnostic yield and clinical concordance of NGS in the diagnosis of a cohort of pediatric patients with bone marrow failure. **Patients and Method:** Patients included were those aged between 0-17 years with a diagnosis of Bone Marrow Failure Syndrome according to the ICD-10 classification codes, who had undergone a genetic study between 2018 and 2022. The information was obtained from the electronic medical records system. Genomic DNA was isolated and quantified through the Qubit™ 3.0 fluorometer. Regions of interest were selected using a hybridization probe that inclu-

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ded the intronic and exonic regions adjacent to the genes included in the panel. Clonal amplification and paired-end sequencing of the selected regions were performed using the Illumina MiSeq™ system. Bioinformatics analysis was performed in alignment with the reference genome (GRCh38). Variants classified as probably pathogenic or pathogenic were confirmed through Sanger sequencing. **Results:** Out of 18 patients included, a genetic diagnosis was achieved through NGS in 5 (27.8%) of them: two cases of Fanconi Anemia, two cases of Dyskeratosis Congenita, and one case of TP53-associated bone marrow failure. Clinical concordance was 100%. Two novel variants were found in the FANCA and PARN genes as causing disease. **Conclusions:** The use of NGS in patients with bone marrow failure identified the etiology in close to a third of patients of our cohort, with higher yield in patients with a clear clinical diagnosis and syndromic features.

Introduction

Bone marrow failure (BMF) is a rare, potentially lethal pathology with many questions about its etiology[1]. It can be divided into inherited and idiopathic . Inherited bone marrow failure syndromes (IBMFS) are a heterogeneous group of disorders characterized by BMF associated with at least some additional somatic disorder[2]. They are characterized by the absence or low numbers of hematopoietic progenitor cells in the bone marrow and are estimated to affect approximately 2/1,000,000 people per year, being more frequent in adolescents and young adults[3]. Approximately 25% of pediatric and 10% of young adult patients with BMF have an inherited etiology[4].

Among the most frequent IBMFS are conditions such as Fanconi anemia (FA), dyskeratosis congenita (DC), Diamond-Blackfan anemia (DBA), Schwachman-Diamond syndrome (SDS), severe congenital neutropenia (SCN), Thrombocytopenia-Absent Radius (TAR) syndrome, and congenital amegakaryocytic thrombocytopenia (CAMT).

In Colombia, we do not have specific data on the prevalence of these syndromes; however, in a study previously conducted at the *Fundación Valle del Lili*, 10 cases were found between 2011 and 2017[5]. In recent years, genetic sequencing techniques and the study of the genetic causes of IBMFS have had remarkable improvements. A notable example is next-generation sequencing (NGS); a set of new technologies that allows massive and simultaneous DNA sequencing. This has significantly expanded the ability to identify the molecular causes of these syndromes and facilitate their timely diagnosis, in order to provide individualized treatment, prognosis, and follow-up. In addition, NGS also allows genetic counseling and contributes to the comprehensive care of these patients[6].

The aim of this study was to analyze the performance and usefulness of NGS in the etiological diagnosis of patients with BMF in a high-complexity healthcare center in southwestern Colombia between 2018 and 2022.

Patients and Method

Participant selection and data collection

Patients between 0 and 17 years of age seen at the *Fundación Valle del Lili* with diagnoses of BMFS according to the codes of the International Classification of Diseases 10th edition (ICD-10), who had undergone genetic study between January 2018 and June 2022, were included. Information was collected from the electronic medical records system. Patients with insufficient clinical information to answer the study objectives were excluded.

Genetic analysis

The sequencing process was performed in the clinical laboratory of the hospital. Peripheral blood samples were collected after signing an informed consent form. Genomic DNA was isolated using the DNeasy® Blood & Tissue Kit (QIAGEN, Hilden, Germany). DNA obtained was quantified using the Qubit® 3.0 fluorometer (Thermofisher Scientific, Waltham, MA, USA). The genomic library was prepared using a Library Preparation Kit (*Sistemas Genómicos*, Valencia, Spain). Regions of interest were selected using a hybridization probe that included intronic and exonic regions adjacent to the genes included in the panel. Clonal amplification and paired-end sequencing of the selected regions were performed using the MiSeq™ System (Illumina, San Diego, USA). Bioinformatic analysis was performed according to the reference genome (GRCh38). A variant was defined as a DNA sequence alteration found in the general population with a frequency of less than 1%. The variants were classified using the guidelines of the American College of Medical Genetics and Genomics (ACMG)[7]. Variants classified as probably pathogenic or pathogenic were confirmed by Sanger sequencing.

The NGS panel used included the following genes: *GFI1*, *SLX4*, *WAS*, *FANCA*, *NBN*, *FANCB*, *JAGN1*, *FANCC*, *FANCD2*, *NHP2*, *TERC*, *XRCC2*, *FANCE*, *NOP10*, *TERT*, *DKC1*, *FANCF*, *RAD51C*, *TINF2*,

ELANE, *FANCG*, *FANCI*, *PALB2*, *FANCL*, *PARN*, *BRCA2*, *FANCM*, *BRIP1*, *RTEL1*, *ERCC4*, *G6PC3*, *VPS45*, and *TP53*, reported to be associated with IBMFS.

Statistical analysis

All the data were analyzed using STATA 14® statistical software. Numerical variables were collected, and the presence of normal distribution was evaluated using the Shapiro-Wilk statistical test. Normally distributed variables were summarized using the mean as the measure of central tendency and the standard deviation as the measure of dispersion. Non-normally distributed variables were summarized using the median as the measure of central tendency and the interquartile ranges as the measure of dispersion. Univariate analysis of qualitative variables was summarized as percentages.

To determine the diagnostic yield, the denominator was considered as the total number of patients who underwent the same genetic test, and the numerator as those patients in whom the genetic test confirmed a genetic etiology.

For clinical concordance, the denominator used was the total number of patients with the clinical diagnosis, and the numerator was the number of patients in whom a genetic alteration compatible with the clinical diagnosis was identified.

Ethical considerations

This study was approved by the institutional ethics committee under registration number 1968 on August 10, 2022.

Results

Sociodemographic and clinical characteristics

18 patients with a clinical diagnosis of IBMFS were included. 61% (n = 11) were male. The clinical manifestations presented around 10 years of age, with a mean time to diagnosis of 4 months. Parental consanguinity and family history of hematologic disease were reported in 17% (n = 3) of cases.

88% (n = 16) of the patients had pancytopenia as the initial paraclinical presentation, which persisted during the disease; Table 1 describes in detail the cell lines involved. Among the non-hematologic clinical manifestations, the most frequent were cutaneous (44%), skeletal (17%), and genitourinary (11%).

The most frequent clinical diagnosis was idiopathic BMF syndrome, followed by FA, and DC in 2 cases, respectively. 13 of the patients were referred to hematopoietic stem cell transplantation (HSCT) (table 1).

Genetic results

The only genetic test performed in this group of patients was the NGS panel for BMF, which had an overall diagnostic yield of 27.8%. The yield was 100% in patients with suspicion of a specific clinical syndrome, in this case, FA and DC. In patients classified as idiopathic, the diagnostic yield of genetic testing was 1/13 (13.2%). For patients clinically classified as another type of BMF, the diagnostic yield was 0 (table 2) (figure 1).

Out of the patients clinically classified as idiopathic, a clear genetic cause was found in only one case, which corresponded to a *TP53*-associated BMF syndrome. The concordance of clinical diagnoses was 100% for DC and FA and no reclassifications of diagnoses were made based on the genetic study.

Identified genetic variants are summarized in Table 3. Of the variants classified as pathogenic or probably pathogenic, the variants c.2446_2447delGCinsTA p.Ala816Ter in the *FANCA* gene and c.88G>A p.Glu30Lys in the *PARN* gene have not been previously described in the literature, and are associated with FA and DC, respectively.

Discussion

NGS refers to a group of technologies that allow massive and simultaneous DNA sequencing. It can be performed through targeted gene panels, where a number of specific genes related to the clinical suspicion are evaluated, through whole exome sequencing, which analyzes all coding regions - or exons - of the genome, or through whole genome sequencing. These types of technologies have been successfully used in the diagnostic approach to patients with IBMFS[8].

In our cohort, the most frequent diagnoses of IBMFS were FA and DC, which is similar to that described in the literature, confirming in all four cases the clinical suspicion. Within IBMFS, the genetic cause is identified in approximately 95% of cases of FA or SDS, while in conditions such as DC or DBA, the diagnostic rates are 50-70%. The use of NGS has permitted the description of new variants associated with IBMFS[9,10].

In this case series, two new variants are reported for the first time. The variant c.2446_2447delGCinsTA p.Ala816Ter in the *FANCA* gene associated with FA is a nonsense variant in a gene where loss of function is a known mechanism of disease. It is absent in population databases and was found on the opposite allele (trans) of a pathogenic variant (PVS1, PM2, PM3). The c.88G>A p.Glu30Lys variant in the *PARN* gene associated with DC is a missense variant. *In-silico* predictors suggest a deleterious effect on the protein, it is absent from populational databases, was found in homozygosity in the patient (PP3, PM2). These variants have not been previously submitted to ClinVar.

Table 1. Sociodemographic and clinical characteristics of pediatric patients with bone marrow failure treated at Fundación Valle del Lili, who underwent genetic testing between 2018-2022.

Characteristic	Total (n = 18)
Age at last follow-up, years	
Median (IQR)	14.5 (11-16)
Range	5-18
Male sex, n (%)	11 (61%)
Age at the time of diagnosis, years	
Median (IQR)	10.9 ± 4.7
Range	3-16.6
SD, n (%)	5 (28)
Parental consanguinity, n (%)	3 (16.6%)
Family history of hematologic disorders, n (%)	3 (16.6%)
Age at onset of clinical manifestations, years	
Mean, SD	10 (6-15)
Range	3 - 16
Hematological characteristics at diagnosis, n (%)	
Single-lineage cytopenia	1 (5.5)
Two-lineage cytopenia	1 (5.5)
Pancytopenia	16 (88.8)
Hematological characteristics during disease course, n (%)	
Single-lineage cytopenia	1 (5.5)
Two-lineage cytopenia	2 (11.1)
Pancytopenia	15 (83.3)
Anemia at diagnosis, n (%)	16 (88.8)
Anemia during disease course, n (%)	15 (83.3)
Leukopenia at diagnosis, n (%)	17 (94.4)
Leukopenia during disease course, n (%)	17 (94.4)
Thrombocytopenia at diagnosis, n (%)	18 (100)
Thrombocytopenia during disease course, n (%)	17 (94.4)
Bone marrow findings, n (%)	
Stromal hyaline degeneration	2
Hyaline and myxoid degeneration of the stroma	1
Iron deposits	1
Bone marrow karyotype, n (%)	
Normal	7/7
No culture growth	1
Not performed	10
Chromosomal fragility, n (%)	
Positive	2 (11.1)
Negative	11 (61.1)
Not available	5 (27.8)
Other phenotypic findings, n (%)	
Skeletal anomalies	3 (16.6)
Neurological disorders	1 (5.5)
Skin and appendage anomalies	8 (44.4)
Genitourinary anomalies	2 (11.1)
Cardiac anomalies	1 (5.5)
Hepatic alterations	1 (5.5)
Clinical diagnosis, n (%)	
Fanconi anemia	2 (11.1)
Dyskeratosis congenita	2 (11.1)
Aplastic anemia associated with autoimmune hepatitis	1 (5.6)
Idiopathic aplastic anemia	13 (72.2)
Indication for genetic testing, n (%)	
Confirm clinical diagnosis	5 (28.8)
Rule out genetic etiology	13 (72.2)
Vital status, n (%)	
Alive	15 (83)
Deceased	3 (17)
Hematopoietic progenitor transplantation, n (%)	13 (72)

Table 2. Performance of genetic testing in patients with bone marrow failure syndromes

Clinical Diagnosis	N	Genetic Diagnosis, n (%)	Clinical-Genetic Concordance		Affected genes
			Yes	No	
Fanconi Anemia	2	2 (100)	2	0	FANCA (2)
Dyskeratosis Congenita	2	2 (100)	2	0	PARN, DKC1
Idiopathic Aplastic Anemia	13	1 (7,6)	0	1	TP53
Aplastic Anemia associated with Autoimmune Hepatitis	1	0	0	0	-

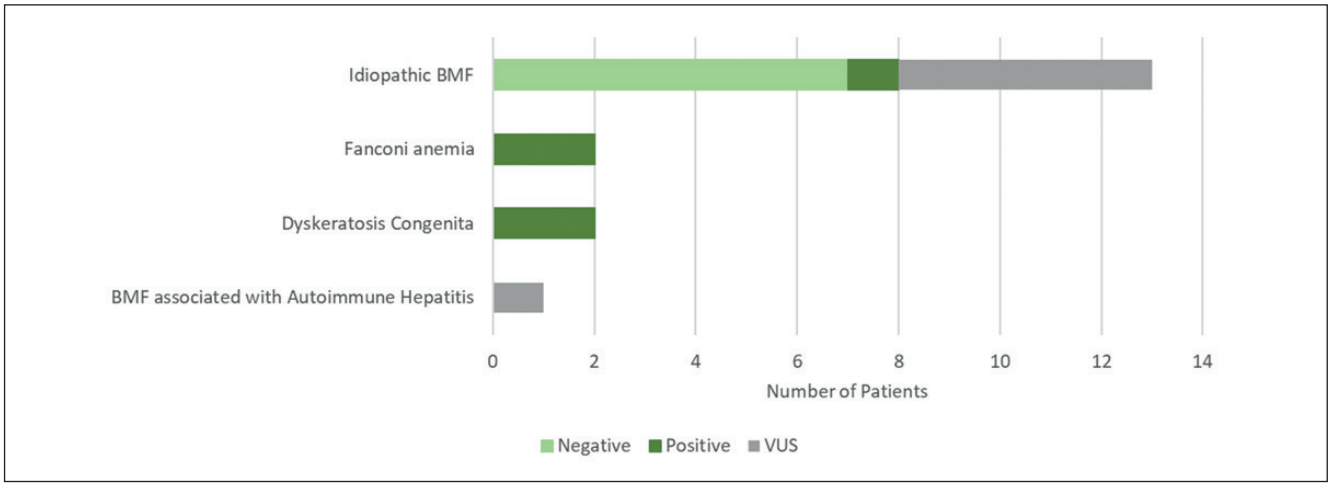

Figure 1. NGS panel results based on clinical diagnosis in pediatric patients with bone marrow failure (BMF: Bone Marrow Failure, VUS: Variant of Uncertain Significance).

Table 3. Genetic findings in patients with inherited bone marrow failure syndromes

Patient	Gene	Variant	Protein Change	Zygosity	Classification
1	FANCA	c.1303C>T	p.Arg435Cys	Homozygous	LP
2	FANCA	c.2446_2447delGCinsTA c.1303C>T	p.Ala816Ter p.Arg435Cys	Heterozygous Heterozygous	LP P
3	PARN	c.88G>A	p.Glu30Lys	Homozygous	LP
4	DKC1	c.1058C>T	p.Ala353Val	Hemizygous	P
5	TP53	c.542G>A	p.Arg181His	Heterozygous	P
6	FANCA	c.1310C>T	p.Ala437Val	Heterozygous	VUS
7	FANCI	c.2505C>G	p.Ser835Arg	Heterozygous	VUS
8	RAD51C	c.640C>T	p.Arg214Cys	Heterozygous	VUS
9	ATM	c.3693_3695delATC c.5917A>G	p.Leu1231_Ser1232delinsPhe p.Arg1973Gly	Heterozygous Heterozygous	VUS VUS
10	NBN	c.1262T>C	p.Leu421Ser	Heterozygous	VUS
11	MSH2	c.2684C>T	p.Pro895Leu	Heterozygous	VUS

VUS: Variant of Uncertain Significance, LP: Likely Pathogenic, P: Pathogenic.

In a patient with a normal phenotype and clinical diagnosis of idiopathic aplastic anemia, a pathogenic variant in the *TP53* gene (c.542G>A) was identified. This variant has been widely described in the literature, associated with an inherited predisposition to cancer, generating a phenotype similar to Li-Fraumeni syndrome[11,12] and functional studies suggest that it alters the function of the *TP53* protein[13]. It has been described that some activating germline variants in the *TP53* are associated with IBMFS, generating a phenotype similar to that of DBA[14].

Additionally, 7 variants of uncertain significance (VUS) were identified in 6 patients, in genes associated with cell damage repair, such as *FANCA*, *FANCI*, *RAD51C*, *ATM*, *NBN*, and *MSH2*. These are variants that lack sufficient evidence to confirm or exclude their role in the clinical presentation of the patient, and therefore should not be used for clinical decision making. The low representation of the Latino population in population-based genomic databases makes it difficult to analyze rare genetic variants, which leads to a higher proportion of variants classified as VUS[15]. These genes are associated with autosomal recessive inheritance phenotypes, requiring the presence of two genetic variants to reach a molecular diagnosis. In the case of patient 11, a variant was found in the *MSH2* gene, involved in the mismatch repair (MMR) pathway. Heterozygous pathogenic variants in this gene are associated with Lynch syndrome, while biallelic variants are associated with constitutional MMR deficiency, a condition characterized by a high risk of hematological malignancies in childhood[16].

In our study, the overall diagnostic yield of NGS-based genetic testing in BMFS was 27.8%, in keeping with what has been reported in the literature (13 to 59%), depending on the selection of patients and the type of molecular study performed[9,17,18]. A study conducted in India that analyzed 42 patients with NGS established a diagnosis in 13% of patients with IAA and 55% of those with a clinical diagnosis of IBMFS[19]. In a Spanish cohort of 201 patients with suspected IBMFS, the overall yield of an NGS panel was 44%, ranging from 24% in patients with unclassified BMF to 48% in patients with clinical suspicion of a specific syndrome[13]. In 121 Japanese patients with a clinical diagnosis of IBMFS evaluated through an 184-gene NGS panel, a diagnostic yield of 44% was obtained[17]. Another study used NGS to find a genetic cause in 48% of 184 patients with BMF with suspected genetic cause but without a clear diagnosis after clinical study and exclusion of FA[20]. An additional study used a 75-gene NGS gene panel that identified the molecular cause in 59% of patients with clinically characterized BMF and 18% of patients with clinically uncharacterized BMF. Of the patients in whom the genetic alteration was found, 20% had changes in their management plan and follow-up, demonstrating the importance and impact of these studies in patient care[18]. In all studies, there were cases of discordance between clinical and molecular diagnosis, which highlights the importance of genetic testing for proper classification of patients.

Genetic testing by NGS has allowed better characterization and more accurate etiological diagnoses in the pediatric population with BMF, thus aiding in the selection of the most appropriate therapeutic strategy. Pediatric patients with IBMFS, although rare, imply a diagnostic challenge due to their high rates of morbidity and mortality, mainly secondary to infectious and hemorrhagic complications, which represent a great social and economic impact. Currently, HSCT is the most frequently used curative option as the first line of management with curative intent. This intervention has shown greater therapeutic success if performed early, ideally within the first 12 weeks from diagnosis. In this case series, 83% of patients were referred for HSCT.

In conclusion, we found a 27.8% diagnostic yield in the cohort of patients with BMF between 2018 and 2022, with a better yield in patients with clear clinical diagnosis and adequate clinical concordance.

Ethical Responsibilities

Human Beings and animals protection: Disclosure the authors state that the procedures were followed according to the Declaration of Helsinki and the World Medical Association regarding human experimentation developed for the medical community.

Data confidentiality: The authors state that they have followed the protocols of their Center and Local regulations on the publication of patient data.

Rights to privacy and informed consent: The authors have obtained the informed consent of the patients and/or subjects referred to in the article. This document is in the possession of the correspondence author.

Conflicts of Interest

Authors declare no conflict of interest regarding the present study.

Financial Disclosure

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