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High frequency of genetic/epigenetic disorders in short stature children born with very low birth weight

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Abstract

Most infants born with very low birth weight (VLBW, birth weight < 1500 g) show spontaneous catch-up growth in postnatal life. The reasons for the absence of catch-up growth are not entirely understood. We performed a comprehensive investigation of 52 children born with VLBW. Ten children had a history of an external cause that explained the VLBW and five refused genetic evaluation. Twenty-three cases were initially evaluated by a candidate gene approach. Patients with a negative result in the candidate gene approach ($n = 14$) or without clinical suspicion ($n = 14$) were assessed by chromosome microarray analysis (CMA) and/or whole-exome sequencing (WES). A genetic condition was identified in 19 of 37 (51.4%) patients without an external cause, nine by candidate gene approach, and 10 by a genomic approach (CMA/WES). Silver–Russell syndrome was the most frequent diagnosis ($n = 5$) and the remaining patients were diagnosed with other rare monogenic conditions. Almost all patients with a positive genetic diagnosis exhibited syndromic features (94.4%). However, microcephaly, neurodevelopmental disorders, major malformation, or facial dysmorphism were also frequently observed in children with an external cause. In conclusion, a significant proportion of children born with VLBW with persistent short stature have a genetic/epigenetic condition.

KEYWORDS
genetics, next-generation sequencing, short stature, very low birth weight

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1 | INTRODUCTION

Infants born with very low birth weight (VLBW) are those with birth weight less than 1500 g, and most of them are born very preterm (VP; gestational age < 32 weeks) and born small for gestational age (SGA, birth weight and/or length ≤ -2SDS) or both. Often, infants born with VLBW have an external factor or a uteroplacental cause that justifies prematurity and/or intrauterine growth retardation (IUGR). Additionally, extrauterine growth retardation due to feed difficulties and acute illnesses contributes to growth deficits during infancy (Wit et al. 2006). Despite these adverse neonatal events, more than 80% of children born with VLBW show spontaneous catch-up growth in postnatal life (Arai et al. 2019; Hollanders et al. 2017). The lack of spontaneous growth recovery is frequently undetermined after usual investigation, including clinical, laboratory, and radiological assessment (Boguszewski et al. 2011; Collett-Solberg et al. 2019a). It is expected that among children born with VLBW without catch-up growth, a genetic cause could explain the observed growth failure (Finken et al. 2018).

Establishing the precise diagnosis for children born with VLBW is especially important for treatment decisions, prognosis, and genetic counseling for the affected patients and their families (Homma et al. 2019; Yaghootkar and Freathy 2012). So then, in the present study, we aimed at investigating the etiology of short stature in a cohort of children born with VLBW without catch-up growth. Our results highlight the importance of clinical recognition of imprinting disorder for the correct molecular-genetic investigation and the use of chromosomal microarray analysis (CMA) and whole exome sequencing (WES) to investigate patients without an apparent external cause explain the low birth weight or the prematurity.

2 | PATIENTS AND METHODS

This study was approved by the Research Ethics Committee of the *Hospital das Clinicas da Faculdade de Medicina da Universidade de of* Sao Paulo (Approval number 37868114.3.0000.0068 in January 27, 2015), and the patients' guardians gave written informed consent. The initial cohort consisted of 52 children born with VLBW referred for short stature investigation in our tertiary care hospital. Patients were considered VLBW with birth weight less than 1500 g according to World Health Organization (WHO) classification. We reviewed the detailed gestational data and collected information regarding neonatal outcomes. Also, we collected information regarding neonatal outcomes, including gestational age, sex, birth weight, length, head circumference, early neonatal complications, drug or surgical treatments, and length of hospitalization. All children underwent a standard evaluation for children with short stature (Collett-Solberg et al. 2019b). We used the Netchine–Harbison clinical scoring system (Wakeling et al. 2017) to select children for Silver–Russell syndrome (SRS) investigation. Anthropometric data were converted to standard deviation score (SDS) using a gender-specific norm (Kuczmarski et al. 2000). The target height was calculated [(father's height + mother's height ± 13 cm)/2] and expressed as SDS.

Genomic DNA was extracted from peripheral blood leukocytes using standard procedures. Fifteen children were clinically diagnosed and submitted to targeted genetic analysis. Patients with clinical diagnosis (score ≥4, $n = 6$) or clinical suspicion (score = 3, $n = 8$) of SRS (Wakeling et al. 2017) were evaluated by Methylation-Specific Multiplex ligation-dependent Probe Amplification (MS-MLPA ME030 BWS/RSS; MRC-Holland). The other nine cases were clinically diagnosed as having monogenic conditions and were evaluated by a targeted panel (Freire et al. 2019). Negative results and patients without clinical suspicion were submitted to CMA and/or WES (Figure 1). Microarray comparative genomic hybridization (aCGH) was performed in a whole-genome 180 K platform (Agilent Technologies, Inc., Santa Clara, CA, USA). Microarray-scanned images were processed using the software Genomic Workbench (Agilent Technologies, Inc.). The parameters used to call a duplication, or a deletion and pathogenicity analysis followed previous studies (Homma et al. 2018). WES was performed with genomic libraries constructed using SureSelect Target Enrichment System (Agilent Technologies, Santa Clara, USA) or xGen Exome Research Panel v2 (Integrated DNA Technologies, Coralville, Iowa, USA). Sequences were generated in the Illumina HiSEQ 2500 or Novaseq 6000 (Illumina, Inc., San Diego, CA, USA) platform running on paired-end mode. Alignment, variant call, and data screening for deleterious single nucleotide variants (SNVs) and small in/dels were performed according to previously published protocols (Freire et al. 2019; Homma et al. 2019; Seo et al. 2020). Copy number variants (CNVs) analysis based on WES data was performed by clinCNV and Delly (Demidov and Ossowski 2019; Rausch et al. 2012). Variant interpretation followed the ACMG/AMP variant pathogenicity guideline (Richards et al. 2015).

Patients were separated into three groups: (1) External cause factor for IUGR/prematurity (maternal or fetoplacental factors); (2) positive genetic cause; and (3) undiagnosed patients. Comparisons were made by ANOVA or ANOVA on Ranks, as appropriate. Nominal variables were compared by Chi-square. Data were analyzed by SigmaStat version 4.0 (Systat Software Inc. Chicago, IL). A p-value <0.05 was considered statistically significant.

3 | RESULTS

We analyzed data from 52 patients born with VLBW referred due to growth disorder. The clinical characteristics of the studied group are presented in Table 1. The median gestational age was 30.5 weeks, ranging from 26 to 39.2 weeks; most were VP (65.4%). The mean birth weight was 1067 g (500–1490) and 44.2% were born small for gestational age. No chronic systemic diseases or endocrine disorders were identified in our whole cohort following the standard assessment for short stature. Five patients refused genetic investigation (Figure 1). Ten patient had an evident history of external cause, as eclampsia ($n = 4$); multiple gestation ($n = 2$); history of maternal substance abuse ($n = 2$); placenta abruption ($n = 1$); or fetal distress ($n = 1$). Among these patients, eight were analyzed by targeted panel sequencing (Freire et al. 2019) and two underwent WES. None

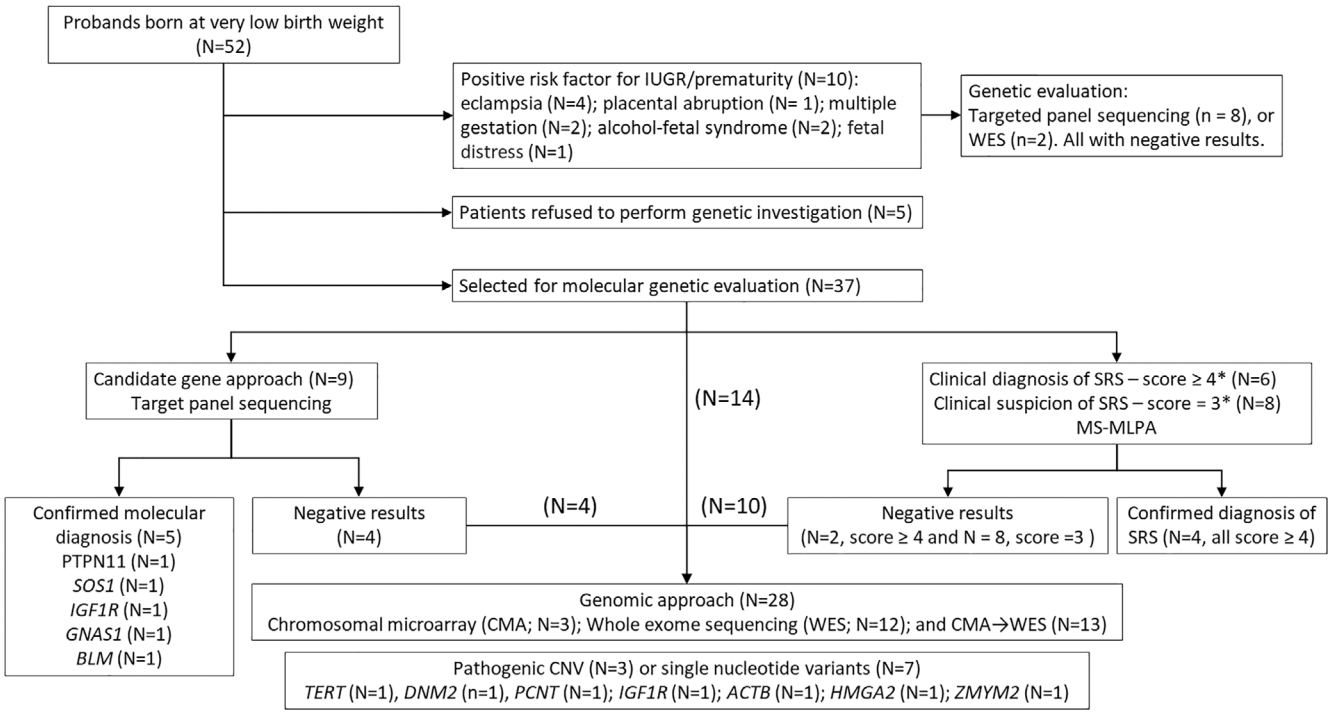

FIGURE 1 Flowchart of the approach used in this study to investigate children with short stature and born with very low birth weight * - Netchine–Harbison clinical scoring system for Silver–Russell (SRS) evaluation (Wakeling et al. 2017).

TABLE 1 Clinical characteristics of a cohort of children born at very low birth weight

	Total patients $N = 52$	External cause $N = 10$	Genetic cause $N = 19$	Undiagnosed patients $N = 23$	P
Sex (m/f)	34/18	7/3	10/9	17/6	0.333
Gestational age (weeks)	30.9 ± 3.6	30.7 ± 3.3	32.1 ± 4.2	30.0 ± 2.9	0.189
Birth weight SDS	−2.1 ± 1.9	−2.6 ± 1.9	−2.0 ± 1.8	−1.9 ± 2.0	0.645
Birth length SDS	−3.3 ± 2.7	−4.6 ± 3.8	−1.9 ± 2.5	−3.9 ± 1.8	0.027[a]
Very premature (%)	34 (65.4)	6 (60.0)	10 (52.6)	18 (78.3)	0.204
Small for gestational age (%)	23 (44.2)	3 (30.0)	9 (47.4)	11 (47.8)	0.601
Age at the 1st evaluation (years)	6.8 ± 5.4	6.5 ± 3.0	7.6 ± 7.0	6.2 ± 4.7	0.707
Height SDS	−3.3 ± 1.5	−3.1 ± 0.9	−3.3 ± 1.3	−3.5 ± 1.8	0.806
Height SDS—target height	−2.2 ± 1.4	−2.0 ± 0.6	−2.3 ± 1.8	−2.1 ± 1.3	0.838
BMI SDS	−1.7 ± 2.0	−1.4 ± 1.8	−2.3 ± 2.1	−1.3 ± 1.9	0.248
Syndromic features (%)	38 (73.1)	5 (50.0)	18 (94.7)	15 (65.2)	0.019
Facial dysmorphism (%)	30 (57.7)	3 (30.0)	12 (63.2)	15 (65.2)	0.142
Neurodevelopmental disorders (%)	22 (42.3)	4 (40.0)	9 (47.4)	9 (39.1)	0.854
Microcephaly (%)	23 (44.2)	3 (30.0)	8 (42.1)	12 (52.2)	0.486
Major malformation (%)	16 (30.8)	1 (10.0)	6 (31.6)	9 (39.1)	0.248
Skeletal dysplasia (%)	2 (3.8)	0	1 (5.3)	1 (4.3)	0.761
Short-stature parent (%)	9/49 (18.3)	2/9 (22.2)	2/18 (11.1)	5/23 (21.7)	0.329
Consanguinity (%)	2/50 (4.0)	0	1 (5.3%)	1 (4.3)	0.772

[a]Student–Newman–Keuls post hoc test: $p = 0.022$ Genetic cause vs. Undiagnosed and $p = 0.059$ Genetic cause vs. External cause.

pathogenic or likely pathogenic variants were identified in these patients with a clear external cause explaining the prematurity or IUGR.

Among the 37 patients without an evident history of external cause, we detected an underlying genetic condition in 51.4% (19/37) (Figure 1 and Table 2). There were no statistical differences among

TABLE 2 Pathogenic variants detected in 19 children born at very low birth weight

ID	Gene/locus	Variant	Zygosity (inheritance)	Final diagnoses (OMIM)
Positive results in candidate gene approach				
1–3	11p15.5	Hypomethylation of *H19/IGF2* IG-HMR	NA	Silver–Russell syndrome (180860)
4	11.p15.5	arr[GRCh37] Arr 11p15.5p15.4 (2029155-2916802)x3	Heterozygous (Inherited from unaffected mother)	Silver–Russell syndrome (180860)
5	*BLM*	exons 20–22 deletion	Homozygous (Autosomal recessive)	Bloom syndrome (210900)
6	*PTPN11*	NM_002834.5:c.218C > T:p.Thr73Ile	Heterozygous (NA)	Noonan syndrome 1 (163950)
7	*GNAS*	NM_080425.3:c.2682C > G: p.Ser894Arg	Heterozygous (de novo)	Pseudopseudohypoparathyroidism (612463)
8	*IGF1R*	NM_000875.5:c.202G > T:p.Glu68*	Heterozygous (Inherited from affected mother)	Resistance to insulin-like growth factor I (270450)
9	*SOS1*	NM_005633.4:c.1297G > A: p.Glu433Lys	Heterozygous (NA)	Noonan syndrome 4 (610733)
Positive results in CMA[a]				
10	4p16.3	arr[GRCh37]4p16.3(49450-2559990) x1	Heterozygous (de novo)	Wolf–Hirschhorn syndrome (194190)
11	13q21.33q34	arr[GRCh37]13q21.33q34 (70141036-113656958)x3 arr[GRCh37]13q34 (113759040-114123122)x1	Heterozygous (NA)	
12	Xq13.1-q13.2	arr[GRCh37]Xq13.1-q13.2 (71470619-73377808)x2	Hemizygous (de novo)	
Positive results in WES				
13	*ACTB*	NM_001101.5:c.616C > T: p.Arg206Trp	Heterozygous (NA)	Baraitser–Winter syndrome 1 (243310)
14	*IGF1R*	NM_000875.5:c.2629C > T: p.Arg877*	Heterozygous (Inherited from affected father)	Resistance to insulin-like growth factor I (270450)
15	*PCNT*	NM_006031.6:c.8413C > T: p.Gln2805* NM_006031.6:c.9099G > C: p.Gln3033His	Compound heterozygous	Microcephalic osteodysplastic primordial dwarfism, type II (210720)
16	*DNM2*	NM_001005361.3:c.1102G > A; p.Glu368Lys	Heterozygous (NA)	Centronuclear myopathy 1 (160150)
17	*HMGA2*	NM_003483.6:c.198 + 1G > C	Heterozygous (Inherited from affected mother)	Silver–Russell syndrome 5 (618908)
18	*TERT*	NM_198253.3:c.2911C > T: p.Arg971Cys NM_198253.3:c.2780 T > G: p.Leu927Arg	Compound heterozygous	Autosomal Recessive Dyskeratosis congenita (613989)
19	*ZMYM2*	NM_197968.4:c.2459-1G > T	Heterozygous (Inherited from her mother)	Neurodevelopmental–craniofacial syndrome with variable renal and cardiac abnormalities (619522)

Abbreviation: NA – not available.

groups (Table 1), except for birth length (patients with a genetic diagnosis had more preserved birth length) and syndromic features, which were more frequently observed in patients with positive genetic findings.

Twenty-three patients had a clinical suspicion of a specific genetic syndrome and were submitted to targeted genetic analysis. SRS was the most suspected condition (OMIM#180860; $n = 14$); the others were clinically diagnosed as Noonan syndrome (OMIM#610733; $n = 3$), Bloom syndrome (OMIM#210900), Resistance to IGF-1 (OMIM#270450), Aarskog–Scott syndrome (OMIM#305400), Coffin–Siris syndrome (OMIM#135900), IMAGE syndrome (OMIM#614732), and pseudohypoparathyroidism (OMIM#612463) (one each). The clinical diagnosis was confirmed by genetic results in five of nine patients suspected of a monogenic syndromic condition. Among patients evaluated for molecular abnormalities of chromosome 11p15.5; four of six patients with clinical diagnosis (score ≥4, $n = 6$) and none of the

patients with clinical suspicion of SRS (score = 3) had a molecular confirmation (Figure 1). Fourteen patients with negative targeted analysis and 14 patients without initial clinical suspicion were selected for genomic approaches. Among these 28 patients, the molecular diagnosis was achieved in 10 cases (36%). Three patients had a pathogenic CNV detected by CMA (a deletion; a duplication and one case with deletion and duplication), and seven had a rare monogenic condition (Table 2): resistance to IGF1 (OMIM#270450), Microcephalic Osteodysplastic Primordial Dwarfism type II (MOPD2) (OMIM#210720), Baraitser–Winter syndrome 1 (OMIM#243310), Centronuclear myopathy (OMIM#160150), Autosomal Recessive Dyskeratosis Congenita 4 (OMIM#613989), Silver–Russell Syndrome 5 (OMIM# 618908); and Neurodevelopmental–craniofacial syndrome with variable renal and cardiac abnormalities (OMIM#619522). All patients had a phenotype compatible with the molecular diagnosis.

4 | DISCUSSION

Nowadays, the leading cause of infant mortality worldwide remains perinatal causes; however, the advances in the management of pregnancy complications and neonatal intensive care have improved survival rates (Crump 2020). Therefore, the number of infants born with VLBW has steadily increased over the years, and a significant adverse outcome is postnatal short stature (Arai et al 2019; Van de Pol and Allegaert 2020). Although the absence of catch-up growth and consequent postnatal short stature may be related to the extrauterine growth retardation, a genetic cause can also be related to postnatal growth disorder (Finken et al. 2018).

We analyzed a group of short-stature children born with VLBW. The etiology of short stature was established in 55.7% of these patients; among them, 35.7% ($n = 10$) had an external evident cause associated with low birth weight/prematurity, and 65.5% ($n = 19$) had a genetic/epigenetic disease (Figure 1). Our findings showed the importance of a complete prenatal and detailed gestational anamnesis; in the absence of a recognized external cause for this condition, the genetic/epigenetic causes represent a major factor associated with VLBW.

Among children with clinical diagnosis or suspicion of a specific condition, the targeted genetic analyses confirmed the initial hypothesis in 39% of the cases. If we excluded the patients selected for chromosome 11p15.5 analyses due to a score of 3 in Netchine–Harbison clinical scoring system for SRS; the confirmation rate rises to 60% of cases selected for candidate gene approach, reassuring the relevance of specialized genetic evaluation before indicating the molecular exam (Mintz et al. 2021). This is particularly important in the recognition of cases of imprinting disorders. Conditions such as SRS and Temple syndrome are associated with intrauterine growth retardation and demand specific molecular-genetic investigation (Wakeling et al. 2017). Indeed, SRS was one of the most frequently established molecular diagnoses in our cohort ($n = 5$, Table 2), with four patients diagnosed during a specific diagnostic test for SRS. Regarding children without a clinical suspicion, the diagnostic rate was 36%, involving chromosomal alteration and a variety of rare monogenic diseases associated with a growth disorder. The diagnostic yield was similar to other studies that evaluated children with growth disorders supporting the use of CMA and WES in clinical practice when evaluating short-stature children born with VLBW (Mintz et al. 2021).

The strengths of this analysis include the evaluation of a relatively large cohort of children born at VLBW with a persistent growth disorder. We applied the current guidelines for clinical and genetic investigation of growth disorders (Collett-Solberg et al. 2019a; Dauber 2019; Finken et al. 2018; Mintz et al. 2021). However, our study had some limitations that should be mentioned. First, patients referred to our tertiary center can represent the most severe cases with a high frequency of genetic/epigenetic disorders. Another potential limiting factor is that not all patients were molecularly evaluated for SRS or had WES/CMA analysis. However, despite these limitations, the present study highlights the importance of clinical investigation linked to genetic tests to establish the diagnosis in this growing group of children with growth disorders.

In conclusion, children born VLBW who do not have catch-up growth have a high frequency of genetic/epigenetic causes, particularly SRS. In the absence of criteria for investigation of SRS or a clear external cause, genetic evaluation using a multigene approach, preferably WES with SNVs and CNVs analysis, allows obtaining the etiological diagnosis in a significant portion of the cases.

AUTHOR CONTRIBUTIONS

Bruna Lucheze Freire, Thais Kataoka Homma and Alexander Augusto de Lima Jorge made substantial contributions to the conception and design, acquisition of data, analysis, interpretation of data, and wrote the first draft of the manuscript. All the authors made substantial contributions to data acquisition and contributed to subsequent drafts.

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CONFLICT OF INTERESTS

Alexander Augusto de Lima Jorge has received consulting fees from NovoNordisk and has an independent research grant from BioMarin. The other authors declare that they have no competing financial interests.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ETHICS STATEMENT

This study was approved by the Research Ethics Committee of the Hospital das Clinicas da Faculdade de Medicina da Universidade de of

Sao Paulo (Approval number 37868114.3.0000.0068 in 27/01/2015), and the patients' guardians gave written informed consent.

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