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Somatic genomic variants in refractory cytopenia of childhood

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ABSTRACT

Despite all knowledge acquired regarding the mutational profile of pediatric myelodysplastic syndrome (MDS), the somatic genomic landscape underlying that disease remains unclear. We evaluated the presence of somatic variants in 37 genes related to myeloid malignancies through targeted NGS in 20 Brazilian patients with refractory cytopenia of childhood (RCC). Only 15% (3/20) of patients showed at least one somatic driver mutation – all in genes coding to regulators of cell signaling (TP53 and CBLB) or epigenetics (ASXL1 and DNMT3A). Interestingly, those variants were identified in patients with no detected clonal chromosomal abnormalities.

Letter to the Editor,

The World Health Organization (WHO) classifies childhood myelodysplastic syndrome (MDS) into refractory cytopenia of childhood (RCC) or MDS with excess of blasts (MDS-EB) [1]. The evaluation of bone marrow (BM) biopsy and aspirate, peripheral blood (PB) smear, and cytogenetics analysis are critical for childhood MDS diagnosis. Moreover, blast counting is needed for its classification [2]. Inherited BM failure syndromes (IBMFS) can predispose children to MDS, and therefore are classified as secondary MDS. However, healthy children either without IBMFS or untreated with chemotherapy previously might develop the malignancy also and account for less than 5% of all pediatric cancers [3].

MDS IBMFS-related and familial MDS/acute myeloid leukemia (MDS/AML) – when multiple individuals within the same family are affected – are commonly associated with germline mutations in *GATA2*, *ETV6*, *RUNX1*, *CEBPA*, *DDX41*, and *SAMD9*/*SAMD9L* genes [4–6]. However, the somatic genomic landscape of childhood MDS is not fully elucidated, and it is under inquiry, especially in *de novo* RCC.

To further investigate the somatic genetic mutations underlying childhood MDS, we employed targeted next-generation sequencing (NGS) comprising of 37 recurrent genes commonly mutated in myeloid malignancies to query 20 cases of primary RCC. This study was carried out in a real-world setting cohort of patients with no history of familial disease *ad hoc* referred to the Brazilian National Cancer Institute (INCA) by two centers of reference in pediatric oncology located in Rio de Janeiro, RJ, Brazil. Clinical data and samples were collected with informed consent from all subjects' parents and approval through institutional human ethics review board-approved protocols from the INCA (CEP/INCA n° 133/12). Patient management and sample collection were conducted in accordance with the Declaration of Helsinki.

DNA samples were isolated from PB or BM using the Qiagen DNA extraction kit according to the manufacturer. Genomic libraries were prepared with the Ion AmpliSeq Customized Panel (ThermoFisher; MA) to evaluate the whole exon-coding and splicing sites of target genes – *AKT1*, *ASXL1*, *ATRX*, *BAP1*, *BCOR*, *CBL*, *CBLB*, *CEBPA*, *CUX1*, *DNMT3A*, *ETV6*, *EZH2*, *FLT3*, *HRAS*, *IDH1*, *IDH2*, *JAK2*, *KIT*, *KRAS*, *MLL*, *MPL*, *NOTCH1*, *NPM1*, *NRAS*, *PHF6*, *PTEN*, *PTPN11*, *RUNX1*, *SF3B1*, *SRSF2*, *SUZ12*, *TET2*, *TP53*, *U2AF1*, *UTX*, *WT1*, and *ZRSR2*. Library sequencing was performed with the Ion Torrent™ Personal Genome Machine™ (PGM) (ThermoFisher; MA). Variants in *GATA2* gene were screened by direct sequencing of exons 2–6 accordingly as previously described [7].

Single-nucleotide variants (SNV) were identified using GATK best practices pipeline developed by the Broad Institute [8], whilst insertion and deletions (INDELs) were analyzed through Ion Reporter Software. Alignment against the GRCh 38 assembly of the human genome was performed. Variants were annotated using the Ensembl Variant Effect Predictor (VEP) [9], and those with less than six supporting reads were filtered out. Finally, in order to identify potential driver mutations, we excluded SNV and INDEL predicted as LOW (synonymous) or MODIFIER (variants in non-translated regions – UTRs; introns) impact, with global minor allele frequency (GMAF) > 0.01 based on 1000 Genome, dbSNP, and gnomAD databases, or when predicted as benign by ClinVar, PolyPhen, or SIFT. Candidate somatic variants were confirmed according to cancer genomic databases, such as The Cancer Genome Atlas (TCGA; NIH) [10], cBioPortal (MSKCC) [11], and Catalogue of Somatic Mutations in Cancer (COSMIC; Wellcome Sanger Institute) [12].

Among the 20 patients investigated, 11 were male and 9 were

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female (ratio 1.2; male/female) with a median age of 5 years (0.3–16). Neutropenia (75%; 15/20) and hypoplasia (65%; 13/20) were the most commonly observed cytopenia in PB and BM cellularity, respectively. Eleven patients had normal karyotype (55%; 11/20), six showed del (17p), −7, or del (7q), and one case harbored complex karyotype (>3 chromosomal abnormalities). Cytogenetic analysis could not be successfully performed in 2 out of 20 cases (10%), and FISH assay did not detect abnormalities in chromosomes 5, 7, 8, and 17 in those samples. Exclusion of other diseases was achieved through serologic testing, investigation of inflammatory and rheumatologic markers, as well as the measurement of immunoglobulins, vitamins, and genetic exams for Dyskeratosis congenita, Diamond-Blackfan anemia, Fanconi anemia, Paroxysmal nocturnal hemoglobinuria, and others. Clinical characteristics and outcomes of patients are shown in Table 1.

NGS targeted 1099 amplicons corresponding to 588 exons and >109K sequenced bases per sample. The average depth of sequencing was 112 reads (range 27–887) and the coverage across the targeted regions was >95% (Supplementary Fig. S1).

Candidate somatic mutations were revealed in *ASXL1, CBLB, DNMT3A,* and *TP53* genes in three patients (Fig. 1; Supplementary Table S1). Interestingly, those variants were identified only in patients with no detected clonal chromosomal abnormalities for harboring normal karyotype. Therefore, the presence of somatic mutations was meaningful to establish clonality in those cases. Co-occurring mutations in *DNMT3A* (c.1225T > C; p.W409R) and *TP53* (c.447dup; p.T150HfsX31) were observed in one case. Higher variant allele fraction (VAF) was associated with *DNMT3A* and *CBLB* mutations (60% VAF). Three out of 20 primary RCC (15%) showed somatic mutations in one (cases RCC06 and RCC17) or two (RCC19) genes. Mutations in *GATA2* (exons 2–6) were screened by direct sequencing in all 20 patients and none presented variants. Patients RCC08, RCC18, and RCC20 transformed into AML approximately 3, 15, 10 months after diagnosis, respectively. RCC08 and RCC18 displayed chromosomal abnormalities at diagnosis, but no somatic variants were observed in the targeted genes. However, the acquisition of other chromosomal aberrations leading to complex karyotypes was confirmed along the leukemic evolution. RCC20 did not show clonality markers either by cytogenetics or NGS during the 20-months of clinical follow-up.

As we searched for somatic mutations in genes implicated to adult MDS and other myeloid neoplasms, we did not evaluate genes with germline variants. No other tissue but hematopoietic was evaluated in our study to assess the genetic background of patients. Nonetheless, it is important to note that all mutations identified in 3 out of 20 cases were described previously as somatic variants

Table 1
Clinical features of the RCC cohort.

Case	Gender (F/M)	Age at diagnosis (years)	BM dysplasia	Cytopenia(s) in PB	Cytogenetics	Mutated genes	Treatment	Follow-up time (months)	Outcome
#RCC01	F	9	Hypolobulated megakaryocytes; erythroid hyperplasia; micromegakaryocytes	N	normal	-	no	105	Alive; persistent cytopenia and BM dysplasia
#RCC02	M	9	Hypolobulated megakaryocytes; micromegakaryocytes; ALIP	N	normal	-	no	73	Alive
#RCC03	F	1	ALIP	N	−7	-	no	115	Alive; spontaneous clinical and cytogenetic remission
#RCC04	F	1	Reduction of granulocytes; micromegakaryocytes	N	del (17p)	-	HSCT	27	Deceased
#RCC05	M	3	Reduction of megakaryocytes; megaloblasts	N	normal	-	no	109	Alive; spontaneous remission of BM dysplasia
#RCC06	F	11	Atypical megakaryocytes; micromegakaryocytes; serous degeneration	N	normal	*CBLB*	no	63	Alive; persistent cytopenia and BM dysplasia
#RCC07	M	3	Dysmorphic granulocytes; ALIP	N	del (17p)	-	no	117	Alive; persistent profound cytopenia
#RCC08	M	1	Reduction of granulocytes; atypical megakaryocytes; micromegakaryocytes	A + N + T	complex	-	Chemo	5	Deceased; progression to AML after 3-months of diagnosis
#RCC09	M	13	Reduction of megakaryocytes; hypolobulated megakaryocytes; erythroid hyperplasia	A + N + T	−7	-	no	26	Alive
#RCC10	M	2	Dysmorphic megakaryocytes and granulocytes	A + T	n/a	-	no	20	Alive
#RCC11	M	15	Erythroid hyperplasia	T	normal	-	no	14	Alive
#RCC12	F	5	Hypolobulated megakaryocytes; micromegakaryocytes; ALIP	A + T	n/a	-	no	91	Alive; trilineage cytopenia after 12-months of diagnosis
#RCC13	M	13	Megaloblasts	N	normal	-	no	39	Alive
#RCC14	F	16	Nuclear-cytoplasmic asynchrony; megaloblasts	T	normal	-	no	97	Alive
#RCC15	F	0	Shift of the M/E ratio; dysgranulopoiesis	N	del (17p)	-	no	98	Alive
#RCC16	M	3	Reduction of megakaryocytes; hypolobulated megakaryocytes; micromegakaryocytes; ALIP	N	normal	-	no	31	Alive; persistent cytopenia and BM dysplasia
#RCC17	F	12	Erythroid hyperplasia; shift of the G/E ratio;	N	normal	*ASXL1*	no	19	Alive
#RCC18	F	8	Erythroid hyperplasia; megaloblasts; ALIP; megakaryocytic hyperplasia	A + N + T	del (7q)	-	Chemo	16	Deceased; progression to AML after 15-months of diagnosis
#RCC19	M	5	Hypolobulated and microcytic megakaryocytes	A + N	normal	*DNMT3A; TP53*	no	75	Alive
#RCC20	M	1	Shift of the G/E ratio; micromegakaryocytes	T	normal	-	Chemo	20	Alive; progression to AML after 10-months of diagnosis

Abbreviations: RCC, refractory cytopenia of childhood; F, female; M, male; BM, bone marrow; ALIP, abnormal localization of immature precursors; PB, peripheral blood; N, neutropenia; A, anemia; T, thrombocytopenia; HSCT, hematopoietic stem cell transplantation; chemo, chemotherapy; AML, acute myeloid leukemia; n/a, not available.

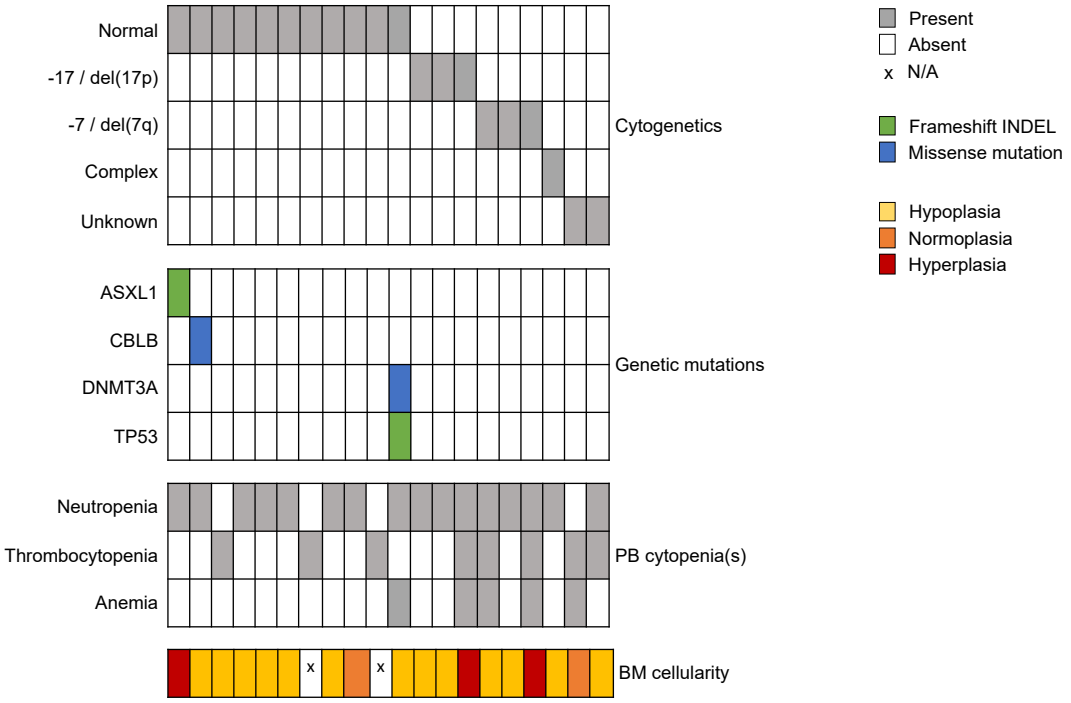

Fig. 1. Genetic alterations in RCC. Chromosome abnormalities, gene mutations, cytopenia(s) in the peripheral blood (PB), and bone marrow (BM) cellularity are indicated in the rows. Each column represents one patient. Frames were colored accordingly to the presence of those characteristics and their respective type.

either in the literature or cancer genomic databases. Unlike pediatric MDS, the comprehensive genomic landscape of adult disease has been broadly investigated across large cohorts of patients. Herein, we observed 15% of cases with RCC has at least one somatic mutation in genes underpin to myeloid malignancies whilst those same markers might harbor somatic variants in up to 80% of adult patients [13,14].

Although cohesin complex coding-genes were not investigated in our NGS panel, the lack of these mutations in pediatric MDS has been reported previously [15]. Also, *SETBP1* mutations are commonly observed in MDS and comprise a recurrent event in patients with abnormalities involving chromosome 7 [16]. However, they were not evaluated using this approach either since those markers were uncovered only after the panel customization.

As observed in our study, *Obenauer* et al. [17] described mutations in genes implicated in epigenetic regulation (*ASXL1*, *DNMT3A*, and others), and transcription factors in pediatric MDS. Whole-exome sequencing (WES) performed by another group suggested the high frequency of mutations in genes related to Ras/MAPK pathway [18]. This may be due to the significant differences in these two cohorts, which have been composed of a higher amount of secondary MDS and JMML cases, in each study, respectively. In addition, *Pastor* et al. [19] showed about 13% of patients with *de novo* RCC harbor somatic mutations in myeloid neoplasms-related genes, in contrast to MDS-EB that showed 2/3 of cases with somatic variants. Even though we described the same frequency of mutations in our cases, no somatic variants were found by them in genes frequently mutated in adult MDS and in age-related clonal hematopoiesis (*DNMT3A*, *TP53*, and others). Furthermore, we did not observe variants in *GATA2* although it was described that 1/3 of patients with germline predisposition were defined by *GATA2* and *RUNX1* mutations in patients with-7/del (7q). Unlike *SAMD9/SAMDL9* genes, variants in *GATA2* may be somatic, mainly when affecting the zinc finger 1 domain [20–22]. This endorses the importance of screening mutations in the gene beside germinative variants. It is worth mentioning that the average depth of NGS sequencing in our study was relatively low. Therefore, it could have led to the lack of detection in variants with low VAF, underestimating potential alterations in our cohort of RCC patients.

Although other groups have proposed the genomic landscape of childhood MDS previously, using broad range approaches such as WES and RNA-seq, our study was the first in Latin America to comprehensively gather and investigate cases with primary RCC and somatic variants in genes implicated to myeloid malignancies (Supplementary Table S2). Besides evaluating a rare subset of patients with very limited data in the literature and underinvestigated especially in developing countries, we described three cases with del (17p). This chromosome disorder, characterized by loss of p53 located on the short arm of chromosome 17, is an important surrogate marker in chronic lymphocytic leukemia (CLL) and multiple myeloma (MM), and is uncommon in childhood MDS [23–25].

Our results confirm previous data and bring to light the novel finds about potential somatic driver mutations of RCC, a pediatric MDS. Somatic variants observed in adult MDS are uncommon in RCC and is a testament to these malignancies as genomically different and are likely driven by different mechanisms. Improving the knowledge about the genetic aspects of pediatric MDS will increase our understanding of the biology of the disease and hopefully will allow earlier diagnosis and establishment of new rational therapies.

Declaration of competing interest

The authors declare no competing interests.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.phoj.2021.04.180.

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Diego F. Coutinho
Centro de Transplantes de Medula Óssea (CEMO), Instituto Nacional do Câncer (INCA), Rio de Janeiro, RJ, Brazil

Mariana Boroni
Centro de Pesquisas, Instituto Nacional do Câncer (INCA), Rio de Janeiro, RJ, Brazil

Anna Beatriz W. Batalha
Instituto de Puericultura e Pediatria Martagão Gesteira (IPPMG), Universidade Federal do Rio de Janeiro (UFRJ), Rio de Janeiro, RJ, Brazil

Danielle T. Vianna
Centro de Transplantes de Medula Óssea (CEMO), Instituto Nacional do Câncer (INCA), Rio de Janeiro, RJ, Brazil

Serviço de Pediatria, Hospital Federal da Lagoa, Rio de Janeiro, RJ, Brazil

Mayara Kalonji, Ana Paula S. Bueno
Instituto de Puericultura e Pediatria Martagão Gesteira (IPPMG), Universidade Federal do Rio de Janeiro (UFRJ), Rio de Janeiro, RJ, Brazil

Soraia Rouxinol
Serviço de Pediatria, Hospital Federal da Lagoa, Rio de Janeiro, RJ, Brazil

Teresa S. Fernandez
Centro de Transplantes de Medula Óssea (CEMO), Instituto Nacional do Câncer (INCA), Rio de Janeiro, RJ, Brazil

Fabiana V. de Mello, Elaine S. Costa
Instituto de Puericultura e Pediatria Martagão Gesteira (IPPMG), Universidade Federal do Rio de Janeiro (UFRJ), Rio de Janeiro, RJ, Brazil

Eliana Abdelhay[1], Bárbara C.R. Monte-Mór[1], Ilana R. Zalcberg[1,*]
Centro de Transplantes de Medula Óssea (CEMO), Instituto Nacional do Câncer (INCA), Rio de Janeiro, RJ, Brazil

[*] Corresponding author. Laboratório de Biologia Molecular, Centro de Transplante de Medula Óssea, Instituto Nacional do Câncer. Praça da Cruz Vermelha, 23, Centro. Rio de Janeiro, RJ, Brazil. CEP: 22230-130.
E-mail address: zalcberg@inca.gov.br (I.R. Zalcberg).

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[1] E.A., B.C.R.M.M, and I.R.Z. share equal credit as last authors.