The identification of *PTPN11* mutations in pediatric myeloid neoplasms in Brazil

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BACKGROUND

PTPN11 mutations are found in myeloproliferative disorders (MD) and acute myeloid leukemia (AML) and are associated with inappropriate activation of RAS/MAPK pathway of myeloid cells. The distinction between MD and AML in childhood is challenging. Diagnostic variables that distinguish MD and AML should be taken in to account, including age strata, blasts morphology, and cytogenetic features. The absence of *BCR-ABL1*, the presence of *PTPN11* mutations and the monosomy of chromosome 7 (mono 7) are found in both MD and AML with Noonan syndrome.

AIM

To identify genetic mutations in RAS/MAPK pathways, with emphasis in *PTPN11*, in order to characterize the clinical and molecular differences of AML and MD.

MATERIAL AND METHODS

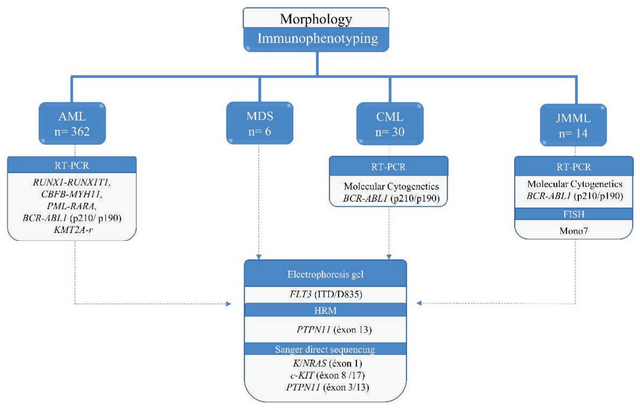

Figure 1. Flowchart of study. Peripheral blood (PB) and/or bone marrow (BM) aspirates of patient samples between 2010-2017 were collected on EDTA at diagnosis. A series of 412 myeloid neoplasms <19 years is the subject of this study [AML (n, 362); JMML (n, 14); CML (n, 30) and MDS (n, 6)]. Initially mononuclear cells were separated by osmotic lysis and marked with monoclonal antibodies to perform the immunophenotyping technique. The identification of AML gene fusions (*RUNX1-RUNX1T1*, *CBFb-MYH11*, *PML-RARa*, *BCR-ABL1* and *KMT2A* rearrangements [*KMT2A-r*]) for characterization of AMLs were performed using FISH, and/or RT-PCR, or multiplex RT-PCR techniques. To screen *PTPN11* gene mutation HRM and Sanger direct sequencing (SdS) were used. Exon 3 were analyzed with SdS while exon 13 was initially analyzed by HRM to identify the mutations. Mono 7 were identified by FISH.

Statistical Analysis

Frequency calculations and univariate analyzes were performed using the χ 2 and Fisher exact tests. Overall survival (OS) estimates were performed using the Kaplan-Meier method and Log-Rank test.

Systematic review

A systematic review (SR) was performed in databases MEDLINE, EMBASE, LILACS and Scopus comparing our results with literature. This review included 26 studies publications, which were classified into three groups: Case report (n = 11), Case series (n =12) and Cohort (n = 3).

RESULTS

Table 1. Demographic characteristics and classification of pediatric AML and MD Brazil, 2010-2017

	n*/n total (%)
Age groups (years)	
≤2	72/412 (17.5)
>2-10	147/412 (35.7)
>11	193/412 (46,8)
Sex	
Males	212/412 (51.5)
Females	200/412 (48.5)
WBC (x10^9/l)	
≤50	212/412 (51.5)
>50	200/412 (48.5)
Geographic regions	
Northeast	163/410 (39.6)
Midwest	121/410 (29.4)
Southeast	101/410 (24.5)
South	23/410 (5.9)
Norte	2/410 (0.5)
* Classificação WHO e CID-O3	
AML with minimal differentiation (M0) 9872/3	19/399 (4.7)
AML without maturation (M1) 9873/3	17/399 (4.2)
AML with maturation (M2) 9874/3	47/399 (11.8)
Acute promyelocytic leukemia (M3) 9866/3	81/399 (20.3)
Acute myelomonocytic leukemia (M4) 9867/3	96/399 (24.0)
Acute monoblastic/monocytic leukemia (M5) 9891/3	52/399(13.0)
Acute erythroid leukemia (M6) 9840/3	5/399 (1.2)
Acute megakaryoblastic leukemia (M7) 9910/3	26/399 (6.5)
Not otherwise specified (NOS) 9861/3	18/399 (4,5)
Chronic myeloid leukemia (CML) 9875/3	17/399 (4.2)
Juvenile myelomonocytic leukemia (JMML) 9946/3	14/399 (3.5)
Myelodysplastic syndromes (SMD) 9895/3	6/399 (1.5)

n+= number of positive cases; WBC, white blood cell; ICD-O3 International Classification of Diseases for Oncology 3rd Edition; WHO, World Health Organization.

Table 2. Distribution of the frequency of fusion genes, molecular aberrations in pediatric AML, CML and JMML according to age and sex, Brazil, 2010-2017

			Age groups (years)				Sex			WBC (x10^9/l)			
Molecular alterations[a]	Frequency n/total (%)	Median (range)	≤2 n (%)	>2-10 n (%)	>11 n (%)	*p*	Males n (%)	Females n (%)	*p*	Median (range)	≤50	>50	*p*
AML	362/412 (87.8)												
RUNX1-RUNX1T1	41/307 (13.3)	8.6 (1.3-20.6)	2 (4.9)	21 (51.2)	18 (43.9)	***0.027***	23 (56.1)	18 (43.9)	0.217	18.4 (3.2-136)	33 (80.5)	8 (19.5)	***0.035***
CBFß-MYH11	18/299 (6.0)	9.45 (1.2-17.8)	2 (11.1)	7 (38.9)	9 (50.0)	0.584	6 (33.3)	12 (66.7)	0.132	73.4 (2.1-373)	5 (27.8)	13 (72.2)	***<0.0001***
KMT2A-r	39/226 (17.2)	1.4 (0.0-21.1)	23 (59.0)	9 (23.1)	7 (17.9)	***<0.0001***	16 (41.0)	23 (59.0)	0.327	35.6 (0.9-251)	22 (57.9)	16 (42.1)	0.128
PML-RARα	55/104 (52.8)	11.2 (1.4-17.9)	2 (3.6)	21 (38.2)	32 (58.2)	0.491	29 (52.7)	26 (47.3)	0.509	9.5 (1.4-190)	41 (74.5)	14 (25.5)	0.030
Mono 7	1/18 (5.6)	[b]	0 (0.00)	0 (100)	0 (0.0)	0.435	1 (100.0)	0 (0.0)	0.722	[b]	1 (100)	0 (0.0)	0.722
FLT3	64/286 (22.4)	11.4 (1,0-21)	1 (1.6)	22 (34.4)	41 (64.1)	***<0.0001***	32 (50.0)	32 (50.0)	0.531	362 (0.9-540)	36 (36.0)	28 (43.8)	***0.037***
KRAS	10/337 (2.9)	13.5 (1.7-15.8)	1 (10.0)	4 (40.0)	5 (50.0)	0.838	5 (50.0)	5 (50.0)	0.621	20.9 (1.0-204)	6 (60.0)	4 (40.0)	0.465
NRAS	24/319 (7.5)	9.9 (0.7-19)	5 (20.8)	8 (33.3)	11 (45.8)	0.782	10 (41.7)	14 (58.3)	0.247	22.1 (8.0-340)	13 (54.2)	11 (45.8)	0.163
KIT	15/193 (7.8)	5.4 (1.3-16.3)	2 (13.3)	9 (60.0)	4 (26.7)	0.225	9 (60.0)	6 (40.0)	0.174	32.9 (4.4-167)	11 (73.3)	4 (26.7)	0.416
PTPN11	22/316 (7.0)	10 (2-18)	2 (9.1)	8 (36.4)	12 (54.5)	0.592	16 (72.7)	6 (27.3)	***0.032***	22.0 (2.3-374)	15 (68.2)	7 (31.8)	0.540
CML	17/412(4.1)												
BCR-ABL1	17/30 (56.6)	15.4 (1.6-20)	1 (5.9)	2 (11.8)	14 (82.4)	0.330	7 (41.2)	10 (58.8)	0.279	294 (4.7-435)	3 (17.6)	14 (82.4)	0.215
JMML	14/412 (3.4)												
Mono 7	2/8 (25.0)	13 (1-4)	1 (50.0)	1 (50.0)	0 (0.0)	0.5133	2 (100.0)	0 (0.0)	0.357	88.7 (38-55)	0 (0.0)	2 (100)	0.750
KRAS	1/10 (10.0)	14 (13-14)	0 (0.0)	0 (0.0)	1 (100)	0.108	1 (100)	0 (0.0)	0.600	3.6 (18-204)	1 (100)	0 (0.0)	0.200
NRAS	1/10 (10.0)	[b]	0 (0.0)	0 (0.0)	1 (100)	0.274	1 (100)	0 (0.0)	0.500	[b]	0 (0.0)	1 (100)	0.900
PTPN11	6/14 (42.8)	12 (0-15)	3 (50.0)	2 (33.3)	1 (16.7)	0.217	4 (66.7)	2 (33.3)	0.262	188 (22-386)	0 (0.0)	6 (100)	0.163

[a]The total number of cases analyzed reflects the availability of biological material for molecular tests; WBC, white blood cell; [b]Median not calculated, ITD, duplications in tandem; TKD, tyrosine kinase domain; AML, leukemia myeloid acute; CML, Chronic myeloid leukemia; JMML, Juvenile myelomonocytic leukemia.

Table 3. Clinical and laboratory characteristics of cases with *PTPN11* mutations

ID	Myeloid neoplasms	Age (months)	Sex	*PTPN11* mutations	Class II alterations	Others alterations of RAS/MAPK pathway
123/10	AML-M0	85	M	c.216 C>T p.A72V	Absent	*NRAS* c. G>T p.G12V
364/10	AML-M1	130	M	c.226 G>A p.E76K	Absent	Absent
395/10	AML-M1	45	M	c.255C>T p.H85H	Absent	Absent
554/10	AML-M4	164	M	c.255C>T p.H85H	Absent	Absent
585/10	AML-M4	205	M	c.227A>C p.A76V	Absent	Absent
071/11	AML-M4	202	M	c.255C>T p.H85H	Absent	Absent
218/11	AML-M4	171	M	c.227A>C p.E76A	Absent	Absent
303/11	AML-M4	95	F	c.255C>T p.H85H	Absent	Absent
456/11	AML-M4	83	M	c.216 C>T p.A72V	Absent	Absent
497/11	AML-M4	41	M	c.226G>A p.E76K	Absent	Absent
260/12	AML-M5	162	M	c.255C>T p.H85H	Absent	*KRAS* c.35 G>A p.G12D
480/12	AML-NOS	21	F	c.255C>T p.H85H	Absent	Absent
323/13	AML-M4	50	M	c.181G>T p.D61y	Absent	Absent
377/13	AML-M3	136	F	c.255C>T p.H85H	*PML-RARα*	Absent
002/14	AML-M4	127	M	c.255C>T p.H85H	*RUNX1-RUNX1T1*	Absent
146/15	AML-M1	168	M	c.223 G>A p.E76k	*KMT2A-r*	Absent
250/15	AML-M0	185	F	c.255C>T p.H85H	*KMT2A-r*	*FLT3-ITD*
301/15	AML-M4	193	M	c.182A>C p.D61A	Absent	Absent
479/15	AML-M5	90	F	c.182 A>T p.D61V	Absent	Absent
0153/10	JMML	44	M	c.214 G>A p.A72T c.255C>T p.H85H	Absent	Absent
0043/13	JMML	183	F	c.255C>T p.H85H	NA	Absent
0042/14	JMML	46	F	c.181 G>T p.D61Y	Absent	Absent
0001/16	JMML	6	M	c.226 G>A p.E76K	NA	Absent
0344/16	JMML	5	M	c.226 G>A p.E76LK	NA	Absent
205/16	AML-M2	211	F	c.215 C>A p.A72D		
024/17	AML-M0	105	M	c.214G>A p.A72T	Absent	Absent
308/17	AML-M3	20	M	c.255C>T p.H85H	*PML-RARα*	Absent
353/17	JMML	14	M	c.214 G>A p.A72T	NA	Absent

ID, identification; WBC, White blood cell; M, male; F, Female; NA, not applicable; AML, leukemia myeloid acute; CML, Chronic myeloid leukemia; JMML, Juvenile myelomonocytic leukemia.

Table 4. Univariate analysis of overall survival in AML and MD, Brazil, 2010-2017

	Univariate analysis			
	N (N of events)	5-year pOS, (SE)	Median ¶, (95% CI)	*p* †
Myeloid neoplasm*				***0.106***
Acute myeloid leukemia (AML)	236 (109)	33.2 (4.4)	15.2 (10.3-20.6)	
Chronic myeloid leukemia (CML)	12(3)	65.5 (16.7)	§	
Geographic regions of treatment *	254 (110)			***0.010***
Northeast	109 (46)	34.3 (6.4)	16.0 (4.2-24.9)	
South	14 (41)	80.0 (12.6)	§	
Southeast	57 (21)	54.5 (7.6)	§	
Midwest	74 (41)	11.0 (8.4)	6.1 (0.6-11.6)	
Age range (years)*	260 (111)			0.203
≤2	60 (22)	35.7 (12.6)	34.1 (6.1-62.2)	
>2-10	92 (36)	33.2 (7.2)	15.4 (11.4-19.5)	
>11	108 (53)	35.5 (5.5)	9.5 (3.8-15.1)	
Race *	260 (111)			***0.024***
White	103 (41)	39.9 (8.3)	41.6 (11.0-71.3)	
Non-Whites	157 (70)	31.1 (5.1)	10,9 (4,9-16,8)	
Sex *	260 (111)			0.712
Males	131 (59)	34.6 (5.8)	15.2 (7.0-23.3)	
Females	129 (52)	37.5 (6.2)	3.9 (8.9-24.3)	
WBC (x10^9/l) *	259 (111)			0.612
≤50	165 (73)	35.9 (5.0)	15.7 (10.2-21.3)	
>50	94 (38)	35.6 (7.4)	5.3 (1.8-22.4)	
Type I mutations *‡				
FLT3	31 (17)	27.6 (10.1)	11.2 (7.2-15.1)	0.727
KRAS	8 (4)	45.0 (18.8)	16.6 (8.6-24.7)	0.722
NRAS	19 (6)	60.3 (12.8)	§	0.197
KIT	12 (4)	53.0 (18.7)	§	0.650
PTPN11	25 (15)	16.1 (9.8)	5.0 (1.6-8.4)	***0.006***
Concomitant type I mutations *	67 (36)			0.250
Single mutation¥	69 (35)	38.5 (6.8)	11.0 (4.7-17.3)	
More than one mutation ¥	6 (5)	16.7 (15.2)	3.0 (0.0- 17.4)	

¶ Median survival in months. † p values from log-rank test indicate whether the differences are significant between the subgroups. * Acute promyelocytic leukemia, AML/MDS and CML with *BCR-ABL1* positive were excluded. ‡Analysis performed between groups positive and negative for the molecular alteration. § Median not reached. CI, confidence interval; N, number; pOS, the probability of overall survival; SE, Standard error. ¥ *K-NRAS*, *FLT3*, *KIT* and *PTPN11*.

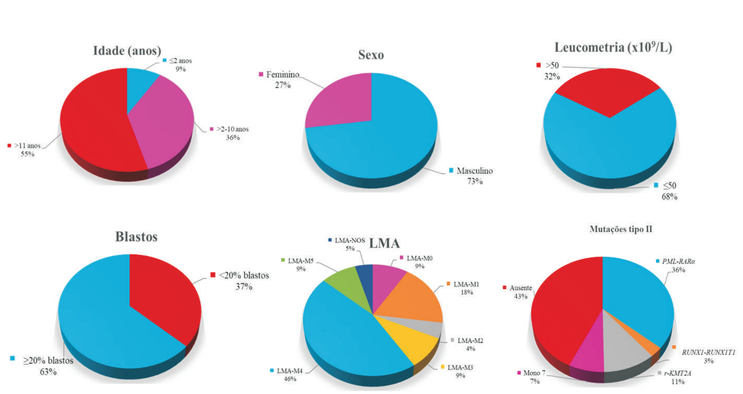

Figure 2. Clinical-laboratory characteristics and molecular aspects of AML cases with *PTPN11* mutations

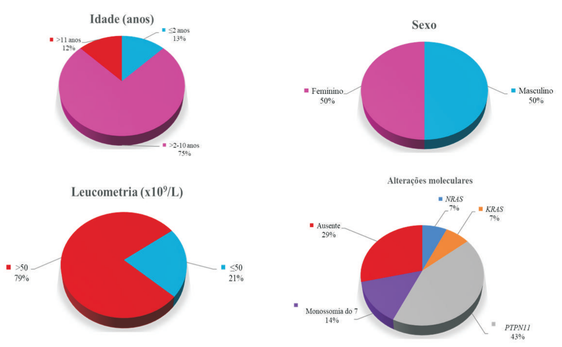

Figure 3. Clinical-laboratory characteristics and molecular aspects of JMML cases

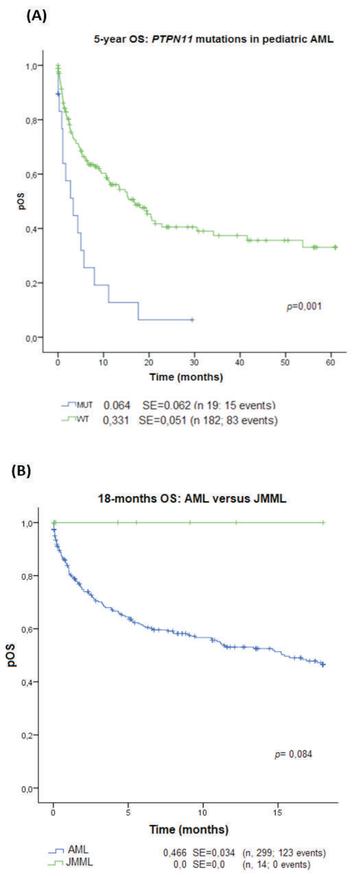

Figure 4. Survival analysis of the pediatric AML cases with mutated *PTPN11* and AML versus JMML. Kaplan-Meier estimates for the probability of overall survival (pOS) for *PTPN11* mutations **(A)** and AML versus JMML **(B)**. p values were calculated using log-rank test. SE, standard error.

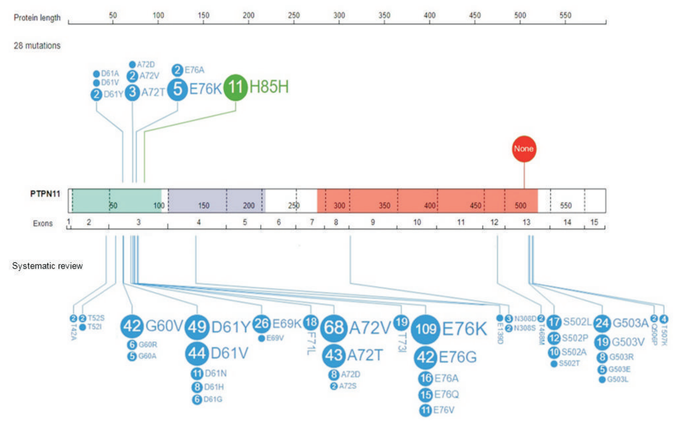

Figure 5. The illustration depicts hot spot regions of *PTPN11* mutations. The occurrence of 28 out of 412 cases tested (6.8%) was observed only in exon 3 were recurrent and codon D61, A72, E76 and H85 that encodes part of N-SH2 domain of protein tyrosine phosphatase. The mutations described by the studies included in SR are shown below the protein.

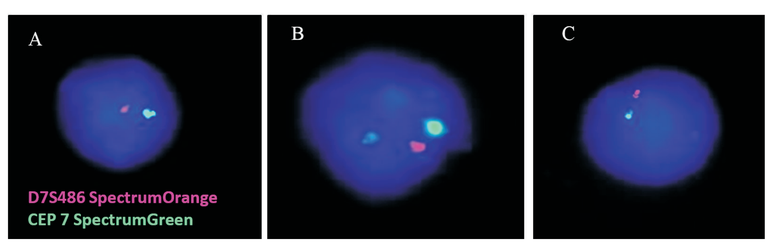

Figure 6. The interphase FISH images show the signals for the chromosome 7 centromeric probe (SpectrumGreen) and 7q31 probe (SpectrumOrange). **(A)**, Case 001/16, male, 6 months old, diagnosed with JMML, the c.226 G>A (E76K) mutations was detected in *PTPN11*. **(B)**, Case 123/10, 7 years old, male, diagnosed with AML-M0, carried c.G>T (G12V) mutation in NRAS and c.216 C>T (A72V) mutation in *PTPN11*. **(C)**, Case 153/10, 3 years old, male, with initial diagnosis of CML-*BCR-ABL1* negative, the identification of *PTPN11* (c.214 G>A [A72T] and c.255C>T [H85H]) mutations led to the diagnosis as JMML.

CONCLUSION

PTPN11 mutations had a significant impact on the overall survival in pediatric AML indicating that these alterations may affect different hematopoietic malignancies.

Projeto Gráfico: Setor de Edição e Informação Técnico-Científica / INCA

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