



LETTER TO THE EDITOR

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# CSF3R, SETBP1 and CALR mutations in chronic neutrophilic leukemia

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## Abstract

The WHO 2008 definition of chronic neutrophilic leukemia (CNL) is based on clinical and laboratory parameters but not on molecular abnormalities. Mutations in *CSF3R*, *SETBP1* and *CALR* are reported in patients with chronic neutrophilic leukemia (CNL). However, because CNL is rare, there are few large studies of this issue. We sequenced these genes in 14 patients who met the WHO-criteria of CNL. 8 subjects had *CSF3R*<sup>T618I</sup>, 6 *SETBP1* mutations and 1 a *CALR* mutation. Our data suggest mutation analysis of *CSF3R*, *SETBP1* and *CALR* should be included in the diagnostic criteria for CNL. These data may also have therapy implications.

**Keywords:** Chronic neutrophilic leukemia, CNL, CSF3R, SETBP1, CALR, Mutation

## To the Editor

The WHO defines chronic neutrophilic leukemia (CNL) as a myeloproliferative neoplasm (MPN) with sustained elevated neutrophils and <10% immature cells [1]. Recently, recurrent somatic mutations in the membrane proximal domain of *CSF3R* were reported in patients with CNL [2,3]. *CSF3R* was mutated in 100% [3], *SETBP1* 33% [3] and *CALR* in 12.5% of WHO-defined cases of CNL [4]. We analyzed mutations in *CSF3R*, *SETBP1* and *CALR* in 14 subjects who met the WHO-criteria.

## Findings

*CSF3R* exon 14–17 [3], *SETBP1* exon 4 [3] and *CALR* exon 9 [5] were amplified by PCR and sequenced. 8 subjects who met the WHO 2008 CNL criteria had a *CSF3R*<sup>T618I</sup> mutation, 6 *SETBP1* mutations (2 D868N, 2 I871T, 1 G870S and 1 D874N) and the last had a *CALR* mutation (c.1154-1155insTTGTC). All mutations were heterozygous except 1 case of *SETBP1*<sup>I871T</sup>. 6 other subjects, 2 with monoclonal gammopathy of unknown significance (MGUS)-associated CNL and 4 with

reactive neutrophilic leukocytosis had no mutation of these genes. No subject had a *JAK2*<sup>V617F</sup> mutation (Table 1).

The consistent association between *CSF3R*<sup>T618I</sup> and CNL in our study is similar to data of Maxson *et al.* [2] and Pardanani *et al.* [3] (Table 2). Tefferi *et al.* [6] suggested including *CSF3R*<sup>T618I</sup> or other membrane proximal *CSF3R* mutations as a criteria for diagnosis of CNL. We also confirmed the high incidence *SETBP1* mutations in patients with CNL. The mutations we detected focused on a hotspot area from D868 to D874 (Table 1). Although these mutations also occur in other hematologic neoplasms such as atypical chronic myeloid leukemia aCML and chronic myelomonocytic leukemia (CMML), analysis of *SETBP1* mutations could help distinguish CNL from reactive conditions such as infection, inflammatory conditions and non-hematologic neoplasms.

Gotlib *et al.* reported *JAK2*<sup>V617F</sup> mutation in a subject of CNL [7]. Lasho *et al.* reported a *CALR* missense mutation in a subject with CNL [4]. We found concurrent *CSF3R*<sup>T618I</sup> and *CALR* frame-shift mutations in 1 subject. The 5 bp insertion into *CALR* exon 9 is reported in *BCR/ABL1*- and *JAK2*-negative MPNs and results in a 1+ base-pair frame-shift with an altered C-terminus.

There is controversy whether co-existence of MGUS and CNL is one or two diseases. The 2 MGUS subjects in our study had no mutation in *CSF3R*, *SETBP1*, *JAK2* or *CALR*. In another study, none of 6 cases of MGUS-

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**Table 1 Clinical characteristics and laboratory variables**

No.	Diagnosis	Gender/Age	Hb (g/L)	WBC ( $\times 10^9/L$ )	ANC ( $\times 10^9/L$ )	PLT ( $\times 10^9/L$ )	Spleen (cm,LCM)	Karyotypes	CSF3R	SETBP1	CALR	JAK2 <sup>V617F</sup>	Treatment	Survival (month)
1	CNL	M/80	120	27.19	25.42	91	3	46,XY,t(1,7)(p32,q11)[10]	T618I	D874N	wt	wt	Hydroxyurea	52
2	CNL	M/64	123	86.83	ND	394	0	46,XY[5]	T618I	D868N	wt	wt	Hydroxyurea	5+
3	CNL	F/77	125	35.68	25.92	351	4	46,XY[20]	T618I	G870S	wt	wt	Hydroxyurea	22+
4	CNL	F/49	104	85.61	79.47	20	6	46,XX[20]	T618I	I871T	wt	wt	Hydroxyurea	13+
5	CNL	M/70	86	146.77	121.82	104	15	ND	T618I	I871T	wt	wt	Hydroxyurea	17
6	CNL	M/43	55	112.65	101.1	98	10	46,XY[5]	T618I	D868N	wt	wt	Hydroxyurea	4+
7	CNL	F/69	102	57.40	40.16	231	6	46,XX[12]	T618I	wt	c.1154-1155insTTGTC	wt	Hydroxyurea	10
8	CNL	M/45	119	32.76	28.73	286	6	46,XY[9]	T618I	wt	wt	wt	Hydroxyurea	32
9	MGUS-CNL	M/46	63	65.30	60.7	101	7	46,XY[2]	wt	wt	wt	wt	Hydroxyurea	6+
10	MGUS-CNL	F/52	121	26.53	19.94	170	3	46,XX[20]	wt	wt	wt	wt	Hydroxyurea	27+

All the parameters in Table 1 were measured at the initial diagnosis in our hospital.

MGUS-CNL: monoclonal gammopathy with uncertain significance associated CNL; Hb: hemoglobin; WBC: White Blood Cell Count; ANC: Absolute Neutrophil Count; PLT: Platelet Count; Spleen (cm): Spleen size under left costa. ND: not done.

**Table 2 CSF3R and SETBP1 mutations in CNL**

	Gotlib J et al. (2013) [7]	Pardanani A et al. (2013) [3]	This series	Total
T618I only	1	5	1	7
T618I + SETBP1	4	4	6	14
Compound CSF3R mutations <sup>a</sup> only	2	1	0	3
Compound CSF3R mutations + SETBP1 mutations	1	0	0	1
Others	1 <sup>b</sup>	2 <sup>c</sup>	1 <sup>d</sup>	4

<sup>a</sup>: compound CSF3R mutations mean nonsense or frameshift mutations that truncate the cytoplasmic tail (truncation mutations) combined with point mutations in the extracellular domain (membrane proximal mutation). In the 3 cases of compound CSF3R mutations Tyner et al. reported, two patients harbored T618I and one harbored T615A in the membrane proximal domain. In Tefferi's study, the compound CSF3R mutation showed T618I + c.2341\_2342insC.

<sup>b</sup>: A case with JAK2 mutation only.

<sup>c</sup>: A case with I598I and a case with M696T in CSF3R.

<sup>d</sup>: A case with CSF3R T618I and CALR frameshift mutation.

associated CNL had *CSF3R* mutations [3]. Also, survival of patients with MGUS-associated CNL is significantly longer survival than those with CNL only. These data support the notion patients with MGUS and CNL are 2 diseases [8].

There may be therapy implications of our findings. *CSF3R* truncation mutations may be sensitive to SRC kinase-inhibitors such as dasatinib whereas *CSF3R* membrane proximal mutations may be sensitive to JAK kinase-inhibitors such as ruxolitinib [9,10]. Ruxolitinib was reportedly effective in a mouse model of CNL and a patient with CNL and a *CSF3R*<sup>T618I</sup> mutation [2,11]. However, ruxolitinib was ineffective in a patient with *CSF3R*<sup>T618I</sup> and *SETBP1* mutations in whom fedratinib suppressed CFU-GM colony formation [12].

#### Competing interests

RPG acknowledges support from the NIHR Biomedical Research Centre funding scheme and is a part-time employee of Celgene Corp., Summit, NJ. The remaining authors declare no competing financial interests.

#### Authors' contributions

XZJ designed the study and drafted the article. CYJ collected the data, analyzed the molecular aberrations, and drafted the article. RPG drafted the typescript. LB, JQ, XZF, QTJ, ZPH, and ZY reviewed the clinical and pathology data. All authors read and approved the final typescript.

#### Acknowledgements

Supported in part by National Natural Science Funds (No.81370611, No. 81270585), Tianjin Key Natural Science Funds (12JCZDJC23900), National Public Health Grand Research Foundation (No.201202017) (to ZX). RPG acknowledges support from the NIHR Biomedical Research Centre funding scheme.

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Received: 10 September 2014 Accepted: 4 October 2014

Published online: 15 October 2014

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doi:10.1186/s13045-014-0077-1

Cite this article as: Cui et al.: CSF3R, SETBP1 and CALR mutations in chronic neutrophilic leukemia. *Journal of Hematology & Oncology* 2014 **7**:77.