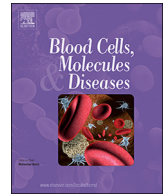




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Letter to the Editor

Concomitant *CALR* and *LNK* mutations leading to essential thrombocythemia with erythrocytosis



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To the editor,

Myeloproliferative neoplasms (MPN) are characterized by a clonal expansion of mature blood cells and mainly include 3 diseases: polycythemia vera (PV), essential thrombocythemia (ET) and primary myelofibrosis (PMF). Their diagnosis has been simplified by the discovery of driver mutations (*JAK2V617F*, *MPL* and *CALR*) that allow detection of a molecular marker in more than 80% of cases. First described in 2005, the *JAK2V617F* mutation is found in more than 96% of PV and around half of ET and PMF. Mutations of *JAK2* exon 12 and *MPL* gene may also, rarely, be found in PV and TE or MPF, respectively. In 2013, a novel type of mutations affecting the *CALR* gene was described in *JAK2V617F* no mutated ET or PMF. *CALR* gene encodes calreticulin, a calcium-binding chaperone protein that promotes folding and quality control in the endoplasmic reticulum. All *CALR* mutations are insertion or deletion localized in the exon 9 that result in a frameshift with a common C-terminal sequence. First described only in ET and PMF, *CALR* frameshift mutations were then found in very few patients with erythrocytosis [1,2]. The mutated protein interacts with *MPL* receptor for *JAK2/STAT5* pathway activation but not with the erythropoietin receptor [3]. When introduced in murine hematopoietic cells, it induces thrombocytosis without erythrocytosis. In a previous report, we identified 2 *CALR* mutated patients within a large cohort of unexplained polycythemia without *JAK2* mutation and reclassified them as (i) ET associated to a minimal polycythemia probably induced by chronic hypoxia and as (ii) an idiopathic erythrocytosis without any clue for MPN [4]. To date, screening for *CALR* mutation for the diagnosis of PV is not advisable and, if such mutation is found, it should not be considered as a convincing explanation for erythrocytosis.

Here we report a case of an 80-years old man that was referred to our lab for a chronic thrombocytosis. The patient had a history of heart arrhythmia and was treated by vitamin K antagonists. The initial examination showed no splenomegaly, mild leukocytosis ($11.9 \times 10^9/L$), thrombocytosis ($902 \times 10^9/L$) and normal hemoglobin and hematocrit (15.2 g/dL and 44.2% respectively). Nevertheless, a low serum erythropoietin (EPO) level (3.5 UI/L) motivated the measure of red cell mass that was increased (126%), without evidence for secondary

erythrocytosis. This argues for the usefulness of the red mass measure in some cases of MPN as previously demonstrated. At the same time a classical molecular screening found no V617F or exon 12 mutation of *JAK2* but a previously described type 2-like mutation of *CALR* (c.1154_1155insATGTC; p.Glu386Cysfs*46; type 33 according Klampfl et al.). To better understand the molecular landscape of this case we performed a 64-genes custom next generation sequencing (Haloplex HS, Illumina NextSeq500) that confirmed the *CALR* mutation with a variant allele frequency (VAF) of 52%. It was associated to a *LNK* mutation (c.639C > A; p.Ser213Arg) with a VAF of 48% that was validated by Sanger sequencing. This mutation of *LNK* occurred in the “hotspot” of the exon 2 coding for the PH domain of the protein and was predicted to be deleterious by both SIFT, Polyphen2 and MutationTaster tools. The Ser213Arg mutation of *LNK* was previously described in a case of *JAK2*-negative erythrocytosis without somatic validation (COSM1685386) [5]. In this previous report the patient had a hemoglobin of 17 g/dL, a hematocrit of 50.4% and a low EPO level (4 UI/L) as in the patient reported herein. This *LNK* variant had a minor allele frequency of 0.063% in gnomAD database (rs111360561). For this reason we controlled the mutation on germinal DNA derived from nails and found that it was indeed a constitutional variant. *LNK* (SH2B3) is an adaptor protein that, after binding, inhibits *JAK2* signaling. *LNK* deficient mice display marked changes of the hematopoiesis, including splenomegaly and a great increase of number of hematopoietic progenitors that have in vitro high proliferative capacities in part due to hypersensitivity to several cytokines [6]. Most of *LNK* mutations identified are missense mutations and occur predominantly in exon 2 coding for the PH domain of *LNK*. *LNK* mutations were found in MPN, especially at leukemic transformation and in idiopathic erythrocytosis (IE) [7]. In particular the mutation Glu208Gln, which is located near the mutation described herein, can be found in both MPN and IE and had a minor allele frequency of 0.099% in gnomAD database.

We therefore retained the diagnosis of *CALR*-mutated ET associated to a *LNK*-mutated constitutional mild erythrocytosis. Bone marrow biopsy was not feasible because of oral anticoagulation. The exclusion of PMF was based on absence of tear drop cells on the blood smear and

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a normal circulating CD34 cells count at 2/ μ L [8]. A treatment by hydroxycarbamide was started with a complete clinicohematological response at 18 months of follow-up.

This report emphasizes the interest of a NGS research for other mutations (such *LNK* or non-V617F *JAK2* mutations) when *CALR* MPN is associated to erythrocytosis. Other causes of erythrocytosis as high oxygen-affinity hemoglobin, *R-EPO* mutations or pathologic EPO production by tumors should also be considered in such cases.

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Ethics approval and consent to participate

The patient was enrolled into the collection “Hémopathies Malignes” of the hospital of Angers who was approved by the CPP (Comité de Protection des Personnes) of Angers (France) Ouest II. A written informed consent was obtained from the patient for the use of clinical and biological data including DNA sequencing.

Competing interests

The authors declare that they have no competing interests.

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