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Received: 6 June 2023 | Revised: 16 October 2023 | Accepted: 18 October 2023

DOI: 10.1111/ejh.14126

European Journal of Haematology

WILEY

CASE REPORT

Extreme thrombocytosis with an aggressive evolution harboring a novel variant of calreticulin (CALR) in exon 3

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Abstract

We describe the case of a patient with extreme thrombocytosis whose evolution was rapidly fatal. No cause of secondary thrombocytosis was found. There was no sign of myelofibrosis but the megakaryocytes were small and dysplastic. The patient presented a calreticulin (CALR) variant in exon 3 (C105S), as well as concomitant mutations of ASXL1, U2AF1, and EZH2. This variant of CALR has never been described before, and after sorting, all identified mutations were found in myeloid cells but not in lymphoid cells. Therefore, the diagnosis of a frontier case of myelodysplastic syndrome/myeloproliferative neoplasm (MDS/MPN) was made. A treatment with hydroxycarbamide was started because of a high risk of thrombosis. Upon worsening of the hematological status two new mutations appeared, SETBP1 and ETV6, and the CALR mutation was still detectable, as well as the three other mutations found in the chronic stage. Our results show that this variant could contribute to MDS/MPN pathogenesis in that patient.

KEYWORDS

acute myeloid leukemia, calreticulin variant, MDS/MPN

Novelty statements

What is the new aspect of your work?

We found a new variant of calreticulin which could be important for pathogenesis of myelodysplastic syndrome/myeloproliferative neoplasm (MDS/MPN) and/or for its transformation in acute myeloid leukemia.

Sarah Bonnet and Serge Carillo contributed equally as co-first authors.

Ludovic Gabellier and Charles Herbaux contributed equally as co-senior authors.

What is the central finding of your work?

The central finding is the discovery of this variant located in exon 3 of calreticulin, which is not explored routinely (unlike exon 9).

What is (or could be) the specific clinical relevance of your work?

This finding could help better characterize some patients with unclassifiable MDS/MPN and more accurately estimate their prognosis.

Thrombocytosis is a frequent condition, which can be due to myeloproliferative neoplasm (MPN) (around 5% of cases) as well as other causes, including infection, trauma or post-surgical state, other cancer or iron deficiency.[1] Extreme thrombocytosis (platelet count ≥1000 G/L) is infrequent; it was reported in less than 2% of patients in a retrospective study involving patients with thrombocytosis. Essential thrombocythemia (ET) is a major etiology of extreme thrombocytosis and most patients with ET harbor a mutation in *JAK2* (60%), *CALR* (20%), or *MPL* (3%).[2] So far, the *CALR* mutations described in ET are frameshift mutations in exon 9.[3] In the other hand, myelodysplastic syndrome/myeloproliferative neoplasms (MDS/MPNs) are disorders in which both dysplastic and proliferative features coexist, which can cause thrombocytosis. The chronic myelomonocytic leukemia (CMML) is the most common MDS/MPN but some cases remain unclassifiable or not otherwise specified (NOS).[4]

We report a case of a 72-year-old female with extreme thrombocytosis. Her past medical history included: hypertension, in situ ductal carcinoma of the right breast treated by lumpectomy and radiotherapy

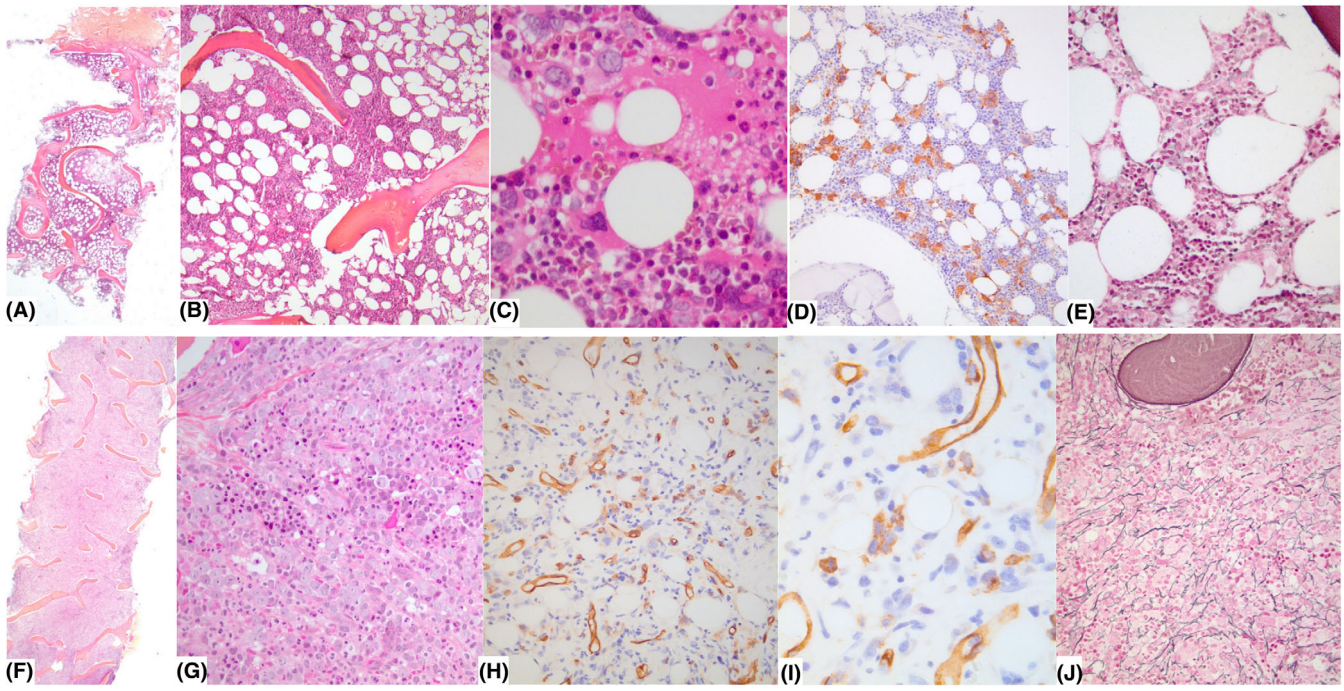

FIGURE 1 Histology of the bone marrow at the time of chronic stage (A–E), and at the time of transformation. (A) Cellularity was globally normal with a homogeneous disposition for age (hematoxylin–eosin saffron (HES 20×). (B) The granulocytic and erythroblastic lineages did not present particular qualitative and quantitative abnormalities and the bone was normal (HES 200×). (C) Megakaryocytes were not in agreement with myeloproliferative neoplasia, many elements were small, dispersed without cluster, no giant form was seen. The nuclei were monolobed without hyperlobulations or bulbous nuclei (HES 400×). (D) By immunohistochemistry (IHC), CD61 antibody confirmed moderate hyperplasia of megakaryocytes with a prevalence of small forms of dysplastic appearance without clusters (CD61 IHC 200×). (E) With Gomori stain, no fibrosis was observed (400×). (F) Cellularity greatly increased for age with a homogeneous density, no bone abnormality was observed (HES 20×). (G) Major hyperplasia of the granulocytic lineage with a maturation blockade. The erythroblastic lineage was hypoplastic made mainly from elements in advanced maturation. Megakaryocytes were small, dysplastic, and less visible compared to diagnosis due to the important granulocytic hyperplasia. They were always dispersed without clusters (HES 200×). (H) By IHC with CD34 antibody, microvascular proliferation was observed without images of extramarrow hematopoiesis. CD34 blasts were present with heterogeneous breakdown. (I) At high magnification several blasts were observed, estimated within 5%–10% according to the medullary spaces analyzed. CD34 blast clusters with four to five cells were frequently visible (CD34 1000×). (J) Medullary fibrosis grade MF2 according to 2022 WHO classification was present (Gomori stain 400×).

(2011) and a ductal carcinoma of the left breast treated by lumpectomy, radiotherapy, and hormone therapy (in complete remission since June 2020). She was referred to hematology department for thrombocytosis, discovered in August 2020 because of an acute left hypochondrium pain. There was no splenomegaly. Blood parameters showed: platelet 1041 G/L, white blood cell (WBC) 18 G/L, ANC 14.4 G/L, monocytes 1.5 G/L, hemoglobin 137 g/L, hematocrit 40.1%, and MCV 90 fL. There was no inflammatory syndrome, no iron deficiency and the hepatic, kidney, and hemostasis tests were normal. She was an ongoing heavy smoker. In her familial history, we had noted a breast cancer (mother), a bowel cancer (grandmother), a kidney cancer (older brother), a marginal zone lymphoma (second older brother). The bone marrow (BM) biopsy showed a normal cellularity, no fibrosis, and the CD34 was negative. The morphology of megakaryocytes was not typical of a myeloproliferative neoplasm. However, the megakaryocyte lineage was moderately hyperplastic and some of the megakaryocytes were small in size with monolobed nuclei as seen in MDSs (Figure 1A–E). The BM aspiration did not reveal any sign of dysplasia, but it was of poor quality with weak density (blood dilution). No cytogenetic analysis was performed at this time. The first molecular biology screening by next generation sequencing (NGS) in August 2020 was negative for *BCR::ABL*, *JAK2* V617F and exon 12, *CALR* exon 9, *MPL* W515L/K/A, and S505N. All molecular biology methods can be found in Supporting Information. A wilder molecular assessment in December 2020 uncovered *CALR* wild type in exon 9 but with a variant in exon 3 (C105S). The variant allele frequency (VAF) of *CALR* C105S was 36.5% and the VAF of *ASXL1* mutation in exon 13 (S392X) of 38.8%. To look for evidence of a non-germline variant of *CALR*, we have sorted WBC of the patient into monocytes, B and T cells. Then we performed a new NGS on each type of cell. The exon 3 *CALR* variant was present in monocytes with a VAF of 38%, whereas it was not detected in lymphoid B cells (VAF 0%) and detected with a VAF of 0.6% in lymphoid T cells. Similarly, the *ASXL1* mutation was found preferentially in myeloid cells (VAF 35.1% in monocytes; 5.7% in B cells; and 3.3% in T cells, Table S1).

Taking all these elements into account, the diagnosis of a frontier case of MDS/MPN was made in March 2021. The WHO criteria for MDS/MPN or MDS were not met because of the lack of a cytopenia, but the cytologic signs of myelodysplasia were clear, as well as the primary nature of the thrombocytosis. Because of the age, the high thrombocytosis and the smoking habit, the risk of thrombosis was considered high, and we started hydroxycarbamide (500 mg/day). The patient was already treated with aspirin. In April 2021, the complete NGS results revealed mutations in *EZH2* W504X (VAF 40%); *ASXL1* S392X (VAF 38%); *U2AF1* Q157P (VAF 39%) and *CALR* C105S (VAF 38%) (Table S2a). In June 2021, the dose of hydroxycarbamide was increased because the platelets rose above 500 G/L. In March 2022, her blood tests showed pancytopenia: Hb 9 g/dL, WBC 3.7 G/L, ANC 1.1 G/L, platelets 121 G/L, she had an anemic syndrome and the hydroxycarbamide was interrupted. In April 2022, she had dyspnea and Grade 3 bone pain. The blood tests showed worsening of anemia (Hb 7.7 g/dL), WBC 3.3 G/L, ANC 1.9 G/L, peripheral blasts 2.5%, and platelets 111 G/L. In this context of MDS/MPN, the appearance of a pancytopenia made us strongly suspect a leukemic transformation. Unfortunately, the BM analyses were challenging to perform because of blood dilution. The BM aspiration uncovered an excess of blasts at 6% in a marrow of very weak density. The flow cytometry analysis was not feasible, while the BM biopsy showed an increased cellularity with evident trilineage dysplasia, excess of blasts around 5%–10% and significant marrow fibrosis assessed at MF2 in accordance with WHO 2022 (Figure 1F–J). Another BM aspiration was also of very weak density, but the flow cytometry highlighted a blast contingent CD34+, HLA DR+, CD33+, CD117+, CD56+ of 15% of cells, consistent with leukemic transformation. The cytogenetic analysis concluded to chromosome 7 monosomy. The NGS showed mutations in *SETBP1* G870S (VAF 4.2%) and D868N (VAF 20.4%), *U2AF1* (VAF 41%), *ASXL1* S392X (VAF 39.9%), *ETV6* Q57X (VAF 9.4%), *EZH2* W504X (VAF 54%), and CALR C105S (VAF 36%), suggesting an onset of *ETV6* and *SETBP1* mutations at transformation (Table S2b). Therefore, the diagnosis of secondary myelofibrosis progressing toward secondary MDS/AML was made. Unfortunately, the clinical status of the patient deteriorated rapidly, with a deep thrombopenia refractory to platelet transfusion. The patient was not fit enough to start an antileukemic treatment and asked for palliative care. She passed away in May 2022.

Here, we report a case of a patient with high thrombocytosis, who harbored a C105S variant of *CALR* which has not previously been reported. Knowing that mutations of *CALR* in the context of MPN are so far all described in exon 9, this novel variant was initially thought to be of unknown significance. However, it was absent from the lymphoid compartment, arguing for its somatic onset. The structure of

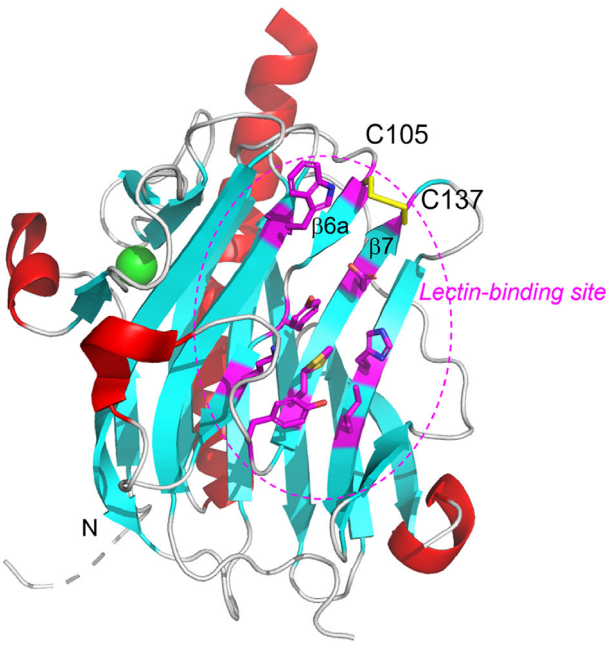

FIGURE 2 Structure of the human CALR globular domain from PDB, entry ID: 3POS. α-Helices and β-sheets are colored in red and cyan, respectively. The Ca2+ ion is represented as a green sphere. The Cys105–Cys137 disulfide bridge, represented as yellow sticks, connects the strands β6a and β7 and stabilized the overall structure. The side chains of the residues involved in the lectin-binding site (including Cys105 and Cys137) are colored in magenta.

human CALR (or CRT) has been published and deposited in the protein data bank (PDB, entry ID: 3POS). The Cys105 forms a disulfide bridge with Cys137 (Figure 2) which stabilized the CALR overall fold and could be involved in CALR chaperone activity, as well as in its binding to carbohydrates and peptides. The variant C105S cannot form this disulfide bridge, cysteine being replaced by serine. Moreover, it has been described that the Cys105 is largely conserved over species and in CALR-related proteins, suggesting a major role in the protein function.[5]

Overall, the absence of any other known etiology for the thrombocytosis, the mutational landscape and the final evolution in AML, lead us to conclude that the patient was presenting a frontier case of MDS/MPN and that the exon 3 *CALR* variant could play a role in the pathophysiology of this disease. Further work will be necessary to precise the functional consequences of this variant.

AUTHOR CONTRIBUTIONS

Barbara Burroni reread the bone marrow biopsies and provided Figure 1. Baptiste Legrand contributed to understand the protein's structure and its potential role in the activity of the protein and provided Figure 2. Serge Carillo performed and analyzed the NGS highlighting the mutation. Guilhem Requirand, Nicolas Robert, and Jérôme Moreaux performed the cell sorting. Sarah Bonnet, Ludovic Gabellier, and Charles Herbaux wrote the manuscript. All authors contributed to, reviewed, and approved this revised manuscript.

ACKNOWLEDGMENTS

The authors would like to thank the patient whose data were collected and reported here, as well as her family.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data are available via email to the corresponding author.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Bonnet S, Carillo S, Legrand B, et al. Extreme thrombocytosis with an aggressive evolution harboring a novel variant of calreticulin (CALR) in exon 3. *Eur J Haematol*. 2024;112(3):475-478. doi:10.1111/ejh.14126