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Myeloproliferative disorders

JAK2 E837K A NOVEL MUTATION POST-POLYCYTHEMIA VERA MYELOFIBROSIS

G. Pasqualini[1|7], M. Giovannini[2], E. De Marinis[3|4], M. Gallo[5], P. Niscola[2], A. Zaza[1|3], E. E. Barroso[1], M. Gentile[3], G. Catalano[1|6], C. Nervi[3], N. I. Noguera[1|6]

[1]IRCCS Santa Lucia Foundation; [2]Hematology Unit, S. Eugenio Hospital ASL Roma 2; [3]Department of Medical and Surgical Sciences and Biotechnologies, University of Rome La Sapienza; [4]Department of Health and Life Sciences, European University of Rome; [5]Department of Medicine and Surgery, University of Parma; [6]Department of Biomedicine and Prevention, Tor Vergata University; [7]Department of Biology and Biotechnology "Charles Darwin", University of Rome La Sapienza, Italy

Mutations in the non-receptor tyrosine kinase JAK2 play a central role in myeloproliferative disorders. JAK2 associates with various cytokine receptors and activates signaling pathways involved in cellular proliferation and survival, including the STAT and PI3K-AKT pathways. Activating mutations, beyond the well-known JAK2V617F, lead to constitutive pathway activation contributing to the myeloproliferative phenotype. This study aims to characterize a novel JAK2E837K mutation discovered in a patient with post polycythemia vera (PV) myelofibrosis (PPV-MF) and to investigate its potential pathogenic role.

The JAK2E837K mutation was identified by Next-Generation Sequencing (NGS) performed on the NextSeq 550® platform using the Archer VariantPlex® panel. A colony-forming unit (CFU) assay was performed on peripheral blood mononuclear cells (PBMCs) from the JAK2E837K PPV-MF patient, 4 healthy donors (HDs) and 8 JAK2V617F PV patients, cultured with increasing concentrations of erythropoietin (EPO). STAT3/5 and AKT activation was assessed by Western blot. Mitochondrial function and glycolytic activity were analyzed using a Seahorse Bioscience XFe96 analyzer.

According to the NCBI Genome Data Viewer and UniProt, the JAK2E837K mutation is located in exon 19, within the interdomain linker connecting the pseudokinase (JH2) and tyrosine kinase (JH1) domains. This linker determines the spatial arrangement between these domains, with JH2 exerting an inhibitory effect on JH1. Phylogenetic analysis showed that residue E837 lies within a highly conserved region across species, suggesting its functional importance (Figure 1A-B). Colony-forming assays revealed that, compared with HDs, the JAK2E837K PPV-MF patient exhibits hypersensitivity to EPO, similar to JAK2V617F PV patients (Figure 1C). Western blot analysis showed increased STAT5 and AKT phosphorylation in colonies from the JAK2E837K PPV-MF patient compared with an HD, whereas no increase in STAT3 phosphorylation was detected (Figure 1D). Finally, the Seahorse analyzer showed increased mitochondrial respiration and glycolytic activity in the JAK2E837K PPV-MF patient compared with the same patient undergoing JAK2 inhibitor treatment and HDs (Figure 1E-F).

In conclusion, we report a novel noncanonical JAK2 mutation in a PPV-MF patient lacking other known driver mutations. JAK2E837K affects a conserved residue within the interdomain linker between JH2 and JH1, likely altering their regulatory interaction. Functional studies demonstrate that the mutation causes constitutive JAK2 activation with increased STAT5 and AKT phosphorylation suggesting a selective activation of the JAK/STAT and PI3K-AKT pathways. Metabolic analyses reveal that JAK2E837K enhances mitochondrial respiration and glycolysis, similar to the canonical JAK2V617F mutation. Overall, these results support the pathogenic role of the JAK2E837K mutation and the contribution of noncanonical JAK2 variants to the pathogenesis of myeloproliferative neoplasms.

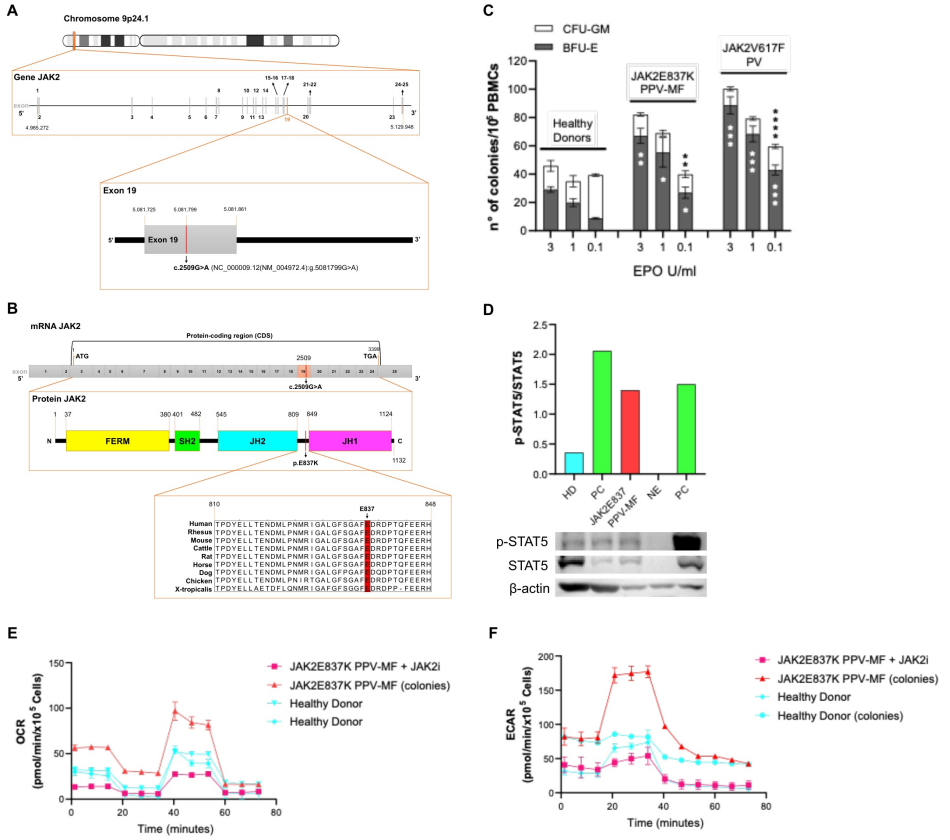

Figure 1. Molecular and functional characterization of the JAK2E837K mutation in a PPV-MF patient.
A, Schematic representation of the chromosomal localization and genomic structure of the human JAK2 gene, including exon 19, according to the NCBI Genome Data Viewer (GRCh38.p14; Chr 9: NC_000009.12) (https://www.ncbi.nlm.nih.gov). All exons are shown in grey, except for exon 19, which is highlighted in orange. Exon 19 is shown in detail, indicating the location of the missense mutation of interest (c.2509G>A, in red). The notation NC_000009.12(NM_004972.4):g.5081799G>A indicates the corresponding genomic position of the JAK2E837K mutation on the JAK2 gene sequence, obtained using Mutalyzer (https://mutalyzer.nl). All positions shown in the figure refer to either the first or last nucleotide of an exon or to the JAK2E837K mutation, based on the coordinates provided by the NCBI Genome Data Viewer. B, Schematic representation of JAK2 mRNA and protein domains, including analysis of evolutionary conservation. All exons are shown in grey, except for exon 19, which is highlighted in orange. Position 1 on the mRNA indicates the first nucleotide of the CDS, corresponding to the start codon ATG. The missense mutation of interest c.2509G>A is shown in red. The schematic representation of protein domains is based on https://www.uniprot.org/; the domains shown are: FERM (in yellow), SH2 (in green), JH2 (in light blue), and JH1 (in pink). The amino acid substitution p.E837K (in red) is located in the interdomain linker connecting the JH2 and JH1 domains. Evolutionary conservation analysis was performed on orthologous proteins from evolutionarily distant species using NCBI Orthologs (https://www.ncbi.nlm.nih.gov). The alignment was performed using https://www.jalview.org/. C, Colony-forming unit assay of PBMCs isolated from 4 healthy donors, the JAK2E837K PPV-MF patient and 8 JAK2V617F PV patients cultured with 3, 1, and 0.1 U/ml EPO. BFU-E are shown as gray bars and CFU-GM as white bars. Data are presented as mean ± SEM. Statistical differences between each group and healthy donors expressing wild-type JAK2 were assessed using Welch's t-test. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$. D, Western blot analysis of protein from JAK2E837K PPV-MF patient's colonies, PBMCs of a healthy donor (HD), positive controls (PC), not evaluable (NE).
E-F, Mitochondrial Oxygen Consumption Rate (OCR) and Extracellular Acidification Rate (ECAR) were measured using a Seahorse Bioscience XFe96 analyzer in the JAK2E837K PPV-MF patient's colonies (in red), in PBMCs from the same JAK2E837K PPV-MF patient undergoing JAK2 inhibitor treatment (in pink), in PBMCs from healthy donors (in light blue) and in colonies from a healthy donor (in light blue).