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# The genomic analysis brings a new piece to the molecular jigsaw of idiopathic erythrocytosis

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## Abstract

Erythrocytosis is a clinical condition characterized by increased red cell mass, hemoglobin, and hematocrit values. A significant fraction of patients is described as having idiopathic erythrocytosis. We have previously demonstrated an association between erythrocytosis and the *JAK2* GGCC\_46/1 haplotype and *CALR* rs1049481\_G allele. In the present study, we investigated genomic and clinical features of 80 erythrocytosis patients with the aim to provide useful information in clinical practice. Patients with idiopathic erythrocytosis could have a genomic germline background, eventually associated with somatic variants. Through association analysis, we show that male patients presenting with idiopathic erythrocytosis, and normal EPO levels could be the best candidates for the search for the *JAK2* GGCC\_46/1 haplotype and *CALR* rs1049481\_G allele. Further studies are needed to confirm these findings and to depict detailed genomic and phenotypical characteristics of these patients.

**Keywords:** Erythrocytosis, Myeloproliferative neoplasms, SNPs, JAK2, EPO

To the editor,

Erythrocytosis is characterized by an erythrocyte count above the gender specific normal range and increased hemoglobin and hematocrit values [1]. Polycythemia vera (PV) accounts for most primary acquired erythrocytosis cases; the *JAK2* V617F or *JAK2* exon 12 variants are

considered PV “driver” mutations. However, about 4% of PV cases lack a molecular marker [1, 2].

Although recent evidence has added useful information to define erythrocytosis [3, 4] a significant fraction of patients is described as affected by idiopathic erythrocytosis (IE), characterized by a genetic marker absence; the IE clinical management still represents an unmet need. We previously demonstrated an association between erythrocytosis and two single nucleotide polymorphisms (SNPs): *JAK2* GGCC\_46/1 and *CALR* rs1049481\_G [5]. In this study, we investigated genomic and clinical features of a larger cohort of patients to unveil the IE molecular complexity (Additional file 1). Based on clinical and genomic data of a more extensive patient’s cohort, we suggest a hierarchical model in which male patients

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presenting with IE and normal erythropoietin (EPO) levels are the best candidates for the search for *JAK2* and *CALR* SNPs. Furthermore, in this subset of patients, we identified additional mutations in genes commonly involved in clonal hematopoiesis (CH).

The *JAK2* and *CALR* SNPs were genotyped in 80 cases (Additional file 2: Table S1) as previously described [5]. Fifty-three (66.3%) were positive and 27 (33.7%) negative for the *JAK2* haplotype. Regarding *CALR*, 54 (67.5%) cases had at least one G allele.

The *JAK2* SNP was associated with erythrocytosis, a significant difference in frequency being detected as compared to healthy European controls ( $p=0.0011$ ). The association was also demonstrated in terms of allelic frequency ( $p=0.0019$ ) and genotype distribution ( $p=0.0035$ ).

The simultaneous presence of both SNPs was observed in 38 (47.5%) cases compared to controls (137/503, 27.2%) ( $p=0.0004$ ). A significant association between SNPs and erythrocytosis was also observed in cases showing normal EPO ( $p=0.0002$ ).

Since both SNPs are in accordance with Hardy–Weinberg equilibrium in controls ( $p>0.05$ ), association analysis was performed between the SNPs investigated and erythrocytosis using the SNPassoc R package [6]. A significant association between *JAK2* SNP and erythrocytosis risk was observed under the dominant model, with a 2.29-fold higher risk in people bearing at least one alternative allele compared to subjects having none (OR = 2.29;  $p=0.0007576$ ) (Table 1). Considering *CALR*, the presence of at least one G allele is associated with

an increased risk under a log-additive model (0,1,2 G: OR = 1.37;  $p=0.06609$ ).

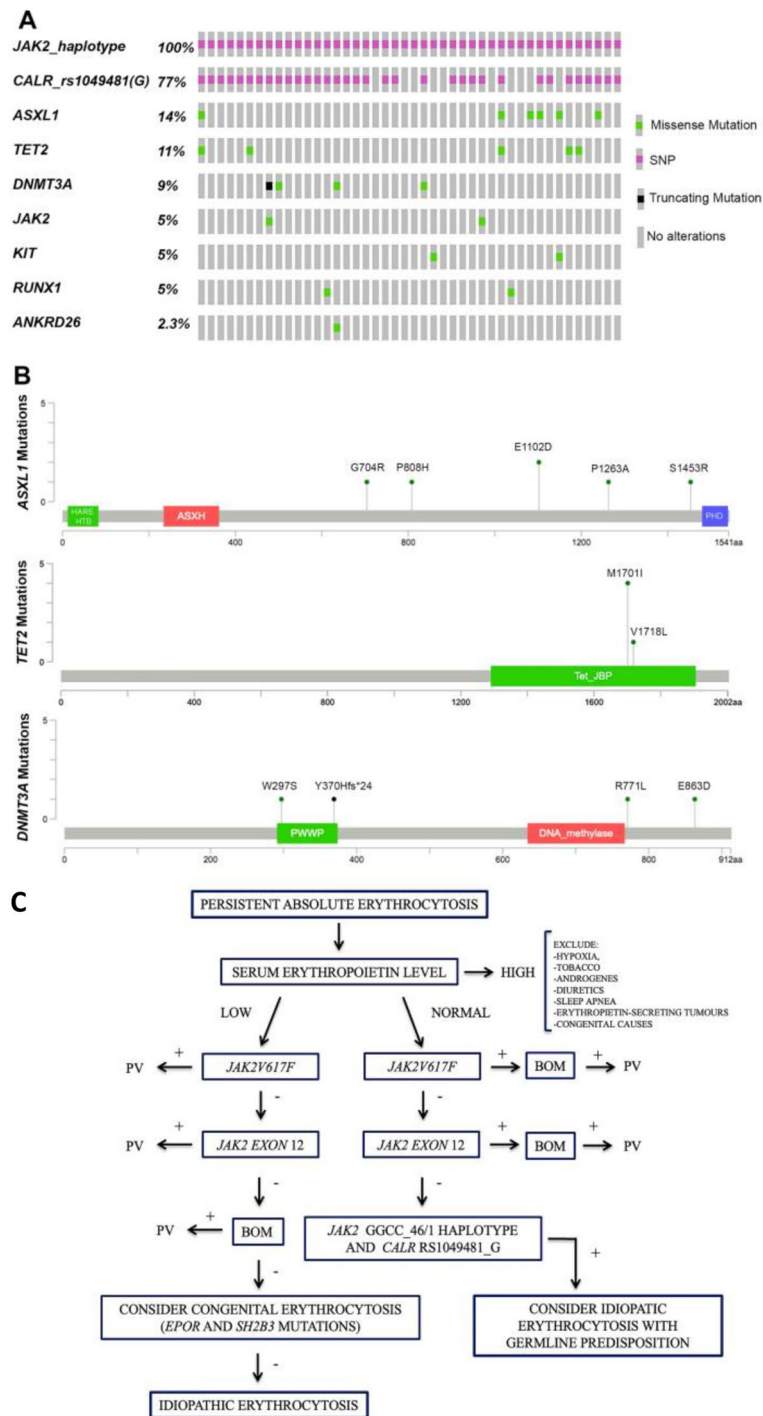
To improve the accuracy of the test, several covariates were incorporated; the association became stronger after adjustment for the presence of *CALR* rs1049481\_G as a categorical variable, as well as gender, and EPO level (Table 1). The erythrocytosis risk is higher when the three covariates are introduced simultaneously (OR = 3.13,  $p=0.000051$ ; Table 1). Considering patients with normal EPO levels, all observed associations between *JAK2* SNP and erythrocytosis under the dominant model were strengthened (with *CALR* rs1049481\_G as covariate: OR = 2.75,  $p=0.0001381$ ; with gender: OR = 3.11,  $p=0.0000522$ ).

Next generation sequencing (NGS) analysis was performed on 44 patients; 34/44 (77%) sequenced cases with the *JAK2* haplotype showed at least one allele G of *CALR* rs1049481. Overall, 22 genetic variants affecting 7 genes (*ASXL1*, *TET2*, *DNMT3A*, *JAK2*, *KIT*, *RUNX1*, *ANKRD26*) were detected in 17/44 cases (38.6%) (Fig. 1A). *ASXL1* was the most frequently mutated gene (6/44, 14%) (Fig. 1A, B). Two non-canonical *JAK2* variants were identified (Additional file 3: Table S2), already described in few patients with haematologic neoplasms [7].

Recent evidence suggests that germline predisposition factors could have a role in the development of myeloproliferative neoplasms [3, 8–10]. Based on the integration of genomic data, clinical features, and statistical methodology, we have attempted to refine the typical characteristics of patients presenting with IE. The median age of our

**Table 1** Associations between *JAK2* GGCC\_46/1 haplotype and erythrocytosis cases

SNP	Genotype	Control (503)	Case (80)
rs3780367	HWE = 0.6868	HWE = 0.4974	HWE = 0.3501
	T/T	271 (53.9%)	27 (33.8%)
	T/G	192 (38.2%)	43 (53.8%)
	G/G	40 (8%)	10 (12.5%)
Genetic inheritance model	OR (95% CI)	p-value	AIC
Codominant	2.23 (1.33, 3.74)	0.0036866	461.1
Dominant	2.29 (1.4, 3.76)	0.0007576	458.9
Recessive	1.65 (0.79, 3.46)	0.1991234	468.6
Overdominant	1.88 (1.17, 3.03)	0.0089603	463.4
log-Additive	1.75 (1.23, 2.47)	0.0019041	460.6
Adjustment by single covariates			
<i>CALR</i> rs1049481_G (yes/no)	2.3 (1.4, 3.78)	0.0007354	459.5
Gender	2.62 (1.54, 4.43)	0.000255	362.9
Epo level	2.73 (1.59, 4.68)	0.000153	417.1
Adjustment by multiple covariates			
Sex-Epo level and <i>CALR</i> rs1049481_G (yes/no)	3.13 (1.76, 5.5)	0.000051	331.9



**Fig. 1** **A** Oncoprint visualization of all genetic variants identified by targeted NGS analysis in 44 erythrocytosis cases. SNP: single nucleotide polymorphism. **B** Maps of the mutations on linear proteins of the most mutated genes in all sequenced cases. Green dots stand for missense mutations, while black dots indicate frameshift mutations. The height of the bar depends on the number of cases bearing each variant. HARE-HTH: HB1, ASXL, restriction endonuclease HTH domain (12–83); ASXH: Asx homology domain (234–362); PHD: PHD domain of transcriptional enhancer, Asx (1480–1539); PWWP: Pro-Trp-Trp-Pro domain (291–374); DNA\_methylase: C-5 cytosine-specific DNA methylase (634–767); Tet\_JBP: Oxygenase domain of the 2OGFeDO superfamily (1290–1905). **C** Diagnostic approach to erythrocytosis patients. PV: polycythemia vera, BOM: bone marrow biopsy

patients with typical CH genes mutations was 52 years (only 2 patients were >60 years). Therefore, such mutations cannot be attributed to an aging-related CH [11].

We hypothesize that a degree of genomic instability could create a “fertile ground” for the development of erythrocytosis, characterized by a high prevalence of additional mutations in typical CH genes. Furthermore, association analysis builds a sort of genomic hierarchy, prioritizing the presence of *JAK2* GGCC\_46/1 over the *CALR* rs1049481\_G allele. Finally, male patients with IE and normal EPO levels are more likely to benefit from the analysis of both *JAK2* and *CALR* SNPs to better define the challenging diagnostic process of IE (Fig. 1C). Further studies are needed to confirm these findings and to depict detailed characteristics of IE patients.

#### Abbreviations

PV: Polycythemia vera; IE: Idiopathic erythrocytosis; SNP: Single nucleotide polymorphism; EPO: Erythropoietin; CH: Clonal hematopoiesis; NGS: Next generation sequencing.

#### Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s40164-022-00301-1>.

**Additional file 1.** Methods.

**Additional file 2: Table S1.** Biological and clinical characteristics of cases analyzed in the present study.

**Additional file 3: Table S2.** Variants identified by NGS analysis in 44 erythrocytosis cases.

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#### Author contributions

Conception and design of the study: AZ, FT and FA. Acquisition of data and/or analysis and interpretation of data: PO, AZ, FT, LA, CC, IR, CFM, NC, GT, AR, IA, EP, MRC, GS, PM and FA. Drafting of the manuscript: FA. All authors revised the manuscript for important intellectual content. All authors read and approved the final manuscript.

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#### Availability of data and materials

The sequence data from this study have been submitted to the National Center for Biotechnology Information (NCBI) Short Read Archive (<https://www.ncbi.nlm.nih.gov/sra/>) under accession number PRJNA609847.

#### Declarations

##### Ethics approval and consent to participate

The local ethics committee approved the study. Informed consent was obtained from all patients before study inclusion, in accordance with the Declaration of Helsinki. Patients’ records/information were anonymized and de-identified before analysis.

##### Consent for publication

Consent for publication was obtained from patients before their enrolment in the present study.

#### Competing interests

The authors declare that they have no competing interests.

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