Genes & Diseases (2024) 11, 561–563

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RAPID COMMUNICATION

Targeted NGS analysis reveals a complex genetic background of idiopathic erythrocytosis in a large Venetian family

Absolute erythrocytosis, due to an increased production of red blood cells, becomes manifest with hemoglobin (Hb) levels above 165 or 160 g/L or with a hematocrit (HCT) above 51% and 48% in males and females, respectively.[1] A minority of patients have polycythemia vera (PV); acquired secondary erythrocytosis frequently occurs due to appropriate or inappropriate erythropoietin (EPO) excess, while congenital secondary erythrocytosis may derive from genetic defects causing tissue hypoxia. Hereditary erythrocytosis occurs in patients with genetic mutations of the oxygen-sensing pathway (*VHL*, *PHD2*, *HIF-1-alpha*) or the erythropoietin receptor (*EPOR*) genes.[2] Recently, *HFE* gene variants have been described in sporadic erythrocytoses.[3] In a high proportion (about 70%) of patients with erythrocytosis, a specific etiology remains elusive despite extensive testing, and the diagnosis of idiopathic erythrocytosis (IE) is ruled out.[1]

A targeted NGS panel for patients with unexplained erythrocytosis was set up. The panel included the coding sequence of *ASXL1*, *BPGM*, *EGLN1*, *EPAS1*, *HAMP*, *FTL*, *HFE*, *HFE2*, *JAK2*, *SLC11A2*, *SLC40A1*, *TFR2*, *VHL*, *FTH1*, and *EPOR* genes involved or suspected to be involved in erythrocytosis. The identified variants were confirmed by direct Sanger sequencing.

We report here the molecular characteristics of a large Venetian family with some cases of erythrocytosis. The genealogic tree of the family is displayed in Figure 1. The patients' main clinical and laboratory data are reported in Table S1.

The proband was a 24-year-old man who had suffered lymphoblastic acute leukemia (LAL) when he was 18 years old and had recovered with chemotherapy. Five years after the end of treatment, erythrocytosis (Hb = 173 g/L, HT = 50%) was identified with no significant alterations in the number of leucocytes and platelets and was associated with high serum ferritin (1421 ug/L). The *JAK2*V617F and rarer *JAK2* exon 12 mutations were searched but not found. The patient had no signs or symptoms of erythrocytosis and phlebotomies were performed to reduce ferritin levels. Recently, the patient developed MDS/MPN-u, and a matched unrelated donor (MUD) hematopoietic stem cell transplantation was performed. The NGS study showed that together with somatic *ASXL1*R1068* mutation (c.3202C > T, NM_015338), the patient carried germinal *EGLN1*C127S (c.380G > C, NM_022051) and *JAK2*L393V (c.1177C > G, NM_004972) variants. The *ASXL1* variants are considered pathogenic and responsible for epigenetic modifications likely to be one of the first events in the tumorigenesis process; it is found in chronic myelomonocytic leukemias, atypical chronic myeloid leukemias, and unclassified myelodysplastic syndromes/myeloid neoplasms. While *JAK2* somatic mutations are well-known driver mutations in PV and other myeloproliferative neoplasms (MPN), the significance of germinal modifications of *JAK2* remains to be elucidated. The *JAK2*L393V germinal variant has been previously described in one patient who had documented normal RBC counts for decades before overt PV became manifest.[4] The functional significance of this variant and its putative predisposing role to PV remain unclear, though its potential to predispose to malignancy is suspected, as the germline *JAK2* variant is frequently found in diffuse large B-cell tumors.

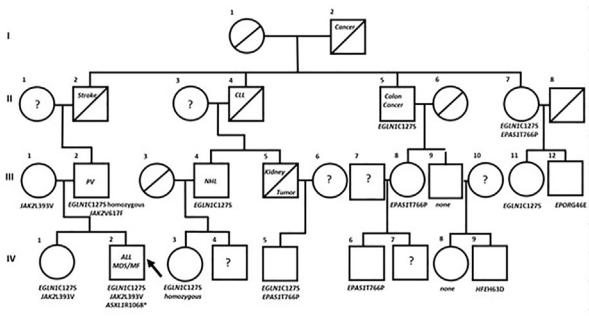
The personal history of the proband revealed that his father (III.2) was affected by PV, confirmed by the presence of the *JAK2*V617F somatic mutation (c.1849G > T, NM_004972). He carried also the homozygous *EGLN1*C127S variant but no relations between *EGLN1*C127S and blood malignancies have been reported to date. No alterations in epigenetic genes were observed.

Given the presence of genetic alterations linked to hereditary erythrocytosis in the proband and his father, we studied other 14 subjects of the family founding some

Peer review under responsibility of Chongqing Medical University.

https://doi.org/10.1016/j.gendis.2023.03.030

A)



B)

	Sex/age	WBC x10^9/L	Hb g/L	HT %	Plts x10^9/L	Clinical data	Molecular variants
II.5	M/94	5.11	123	35.7	209	Ruddy complexion, colon cancer, stroke	EGNL1C127S
II.7	F/86	4.95	105	33.7	201		EGNL1C127S/EPAS1T766P
III.1	F/57	4.69	140	40.4	207		JAK2L393V
III.2	M/66	11.4	187	55.8	488	Ruddy complexion, PV	EGNL1C127S homozygous JAK2V617F (somatic)
III.4	M/68	5.1	153	44	126	NHL	EGNL1C127S
III.8	F/61	5.95	132	38.9	285		EPAS1T766P
III.9	M/57	6.75	150	44.4	340		none
III.11	F/57	6.05	143	42.8	250		EGNL1C127S
III.12	M/52	8.18	139	45.3	254		EPORG46E
IV.1	F/31	7.65	143	44.6	362		EGNL1C127S/JAK2L393V
IV.2 Proband	M/24	6.0	173	49.8	138	LAL-> MDS/MF	EGNL1C127S/JAK2L393V ASXL1R1068* (somatic)
IV.3	F/33	5.36	126	37.8	238		EGNL1C127S homozygous
IV.5	M/45	8.72	149	46.2	224	Ruddy complexion	EGNL1C127S/EPAS1T766P
IV.6	M/34	5.2	195	57.1	149		EPAS1T766P
IV.8	F/15	14.89	137	42.5	338		none
IV.9	M/17	12.03	161	48.8	355	Ruddy complexion	HFEH63D

C)

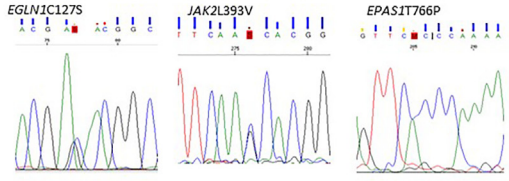


Figure 1 Detailed description of the Venetian family with erythrocytosis. **(A)** Kindred of our Venetian. **(B)** Main clinical and hematologic data of the studied subjects. **(C)** Sanger sequencing of the most representative variant. Hb, hemoglobin; HT, hematocrit; LAL, lymphocytic acute leukemia; MDS/MF, myelodysplastic syndromes/myeloid neoplasm; NHL, non-Hodgkin lymphoma; Plts, platelets at the molecular study time; PV, polycythemia vera; WBC, white blood cell; y, years.

molecular alteration in 12 of them. Elevated or borderline high Hb and HT levels were found in 3 males (IV.5, IV.6, and IV.9), and an associated ruddy complexion was observed in 2 of them. None of the evaluated females had erythrocytosis. In this respect, it is known that IE is more common in males and the present data suggest that females' genetic pattern could be transmitted to their progeny who may eventually develop IE, particularly if of male gender. This could be the case of patients III.8 and IV.6.

Neither subjects carrying gene variants nor those without alterations had a history of thrombotic complications. Yet, the thrombotic risk in IE is higher than that of the general population (1.5/100 patients/year),[5] and mainly patients with overt erythrocytosis need to be adequately followed to prevent the occurrence of cardiovascular events.

Four patients had developed malignancies: one chronic lymphocytic leukemia (II.4), one kidney tumor (III.5), one non-Hodgkin lymphoma (NHL) (III.4), and one colon cancer (II.5). Among these 4 patients, we could study only patients III.4 and II.5; both carried the *EGLN1*C127S variant as 4 other asymptomatic family members (II.7, III.11, IV.1, IV.3, IV.5). Thus, the *EGLN1*C127S gene variant was the most common in our kindred. Such mutation is typical of Tibetans and protects them against erythrocytosis, hypoventilation, and pulmonary hypertension occurring at high altitudes. At normoxic conditions and in low-landers, the *EGLN1*C127S variant is present in 15%—30% of non-Tibetan controls, though its function remains unclear. This variant is relatively common in North-East of Italy and it may represent a genetic inheritance from the barbarian invasions of people coming from the remote areas of central Asia that, once arrived in our neighborhood, intermixed with the resident population.

The germ line *JAK2*L393V variant was found in proband's mother (III.1) and sister (IV.1) who had also *EGLN1*C127S. These patients are being closely followed up so that the eventual appearance of PV is promptly recognized.

Four patients (III.8, IV.6) carried the *EPAS1*T766P (c.2296A > C, NM_001430) variant, which in two cases (II.7, IV.5) was associated with the above-mentioned *EGLLN1*C127S. Patient IV.6 had very high Ht (57.1%) and Hb (195 g/L) levels. The *EPAS1*T766P variant is often considered benign or of uncertain significance, though occasionally has been found to be associated with pheochromocytoma and/or paraganglioma (PPGL).

The *HFE*H63D variant (c.187C > G, NM_000410), present in a single ruddy member (IV.9) of the family, seems not to influence the normal activity of hepcidin but it could play a role in patients with increased hematocrit levels. Finally, the *EPOR*G46E (c.137G > A, NM_000121) variant was observed in an acquired relative (IV.6). The alteration was possibly inherited from his father who used leeches to treat his condition. Such a variant is considered benign and, at any rate, only of accessory relevance in the study of this family.

In conclusion, the NGS study of this family shows the frequent coexistence of multiple mutations of genes involved in erythrocytosis and provides a valuable approach to identifying and eventually clarifying the role of complex multiple molecular mutations occurring in erythrocytosis. Moreover, NGS studies allow identifying specific asymptomatic subjects with erythrocytosis, as they deserve to be carefully followed up to prevent the potential cardiovascular complications of this disease.

Author contributions

All authors participated in the clinical diagnosis of the patients and sample testing. IB and MLR performed the

research, analyzed the data, and wrote the paper. AB and GC prompted the NGS panel and performed NGS evaluations. DR performed molecular biology assays to identify mutations. FP and BG followed the patients and collected their data. All authors revised the paper.

Conflict of interests

The authors have no conflict of interests to declare.

Funding

The paper was partially funded by the Dotazione Ordinaria Ricerca (DOR) - University of Padua.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.gendis.2023.03.030.

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25 November 2022
Available online 18 May 2023