Erythrocytosis associated with *EPAS1(HIF2A)*, *EGLN1(PHD2)*, *VHL*, *EPOR* or *BPGM* mutations: the Mayo Clinic experience

Germline mutations in the oxygen-sensing pathway (*VHL-HIF2A-PHD2*) or erythropoietin (EPO) signaling (*EPOR*) are relatively rare but may result in erythrocytosis with normal p50 measurement (oxygen tension at which hemoglobin is 50% saturated) accompanied by either an elevated or inappropriately normal EPO (*VHL-HIF2A-PHD2*) or subnormal EPO (*EPOR*).[1] On the other hand, a left shift of the oxygen dissociation curve, with venous p50 <24 mmHg may result from high-oxygen affinity (HOA) hemoglobin variants, defective 2,3-bisphospho-glycerate mutase (BPGM) causing 2,3-BPG deficiency or methemoglobinemia.[1] The incidence, clinical course and management of hereditary erythrocytosis has not been well-characterized due to its rare occurrence. In that regard, we recently reported on 41 patients with HOA variant associated erythrocytosis; over half of the patients manifested one or more symptoms thought to be related to increased hematocrit while thrombosis was documented in a quarter of the patients.[2] Neither hematocrit level nor active phlebotomy showed significant correlation with either thrombotic or non-thrombotic symptoms, which might have resulted from the limited sample size.[2] In a recent study which included 270 patients with idiopathic erythrocytosis, 1.1% harbored *EPOR* mutations, while pathogenic variants involving genes in the hypoxia pathway were identified in 23% of patients.[3] Accordingly, we share the Mayo Clinic clinical and laboratory experience with hereditary erythrocytosis resulting from genetic alterations in the oxygen-sensing pathway (*VHL-HIF2A-PHD2*), *EPOR* or *BPGM*.

All patients that underwent hereditary erythrocytosis evaluation at the Mayo Clinic over the last 10 years (2012-2021), were retrospectively recruited after obtaining Institutional Review Board approval. Polycythemia vera was excluded with *JAK2* exon 12-15 sequencing. Hereditary erythrocytosis testing was pursued at the Mayo Clinic laboratory utilizing an algorithmic approach which included p50 measurement, serum EPO level (Epo), and DNA sequencing by polymerase chain reaction (PCR) of *EPOR* (exon 8), hypoxia-inducible factor 2 α (HIF2A) encoded by endothelial PASS domain protein 1 (*EPAS1*) (exons 9 and 12), prolyl hydroxylase 2(PHD2) encoded by EGL-9 family hypoxia inducible factor 1(*EGLN1*)(exons 1-5), von Hippel Lindau (*VHL*) (three coding exons and intron/exon boundaries) and *BPGM* (exons 1-4) as detailed in our prior work.[4]

Of 592 patients tested at the Mayo Clinic for *HIF2A/PHD2/EPOR* alterations, 14 pathogenic variants were identified in *HIF2A* (n=6, 1%), *PHD2* (n=3, 0.5%), *EPOR* (n=2, 0.3%), while two of 421 (0.5%) and one of 446 (0.2%) patients harbored *BPGM* and *VHL* variants, respectively. In addition, 22 variants of uncertain significance (VUS) were reported; *EPOR* (n=1), *HIF2A* (n=3), *PHD2* (n=10), *BPGM* (n=2), *VHL* (n=6), resulting in combined (pathogenic + VUS) Mayo Clinic incidence rates of 0.5%, 1.5%, 2.2%, 1% and 1.6% for *EPOR*, *HIF2A*, *PHD2*, *BPGM*, and *VHL* aberrations, respectively.

Table 1 summarizes oxygen-sensing pathway (*PHD2/HIF2A/VHL*) pathogenic variants including clinical course of ten patients with median follow-up of 2 years, (range, 0.2-10 years). *HIF2A* pathogenic variants were noted in six patients; four harbored the heterozygous HIF2A c.1121T>A, p.(Phe374Tyr) alteration in exon 9, previously reported in association with neuroen-

docrine tumors with or without erythrocytosis.[5] A 57-year-old male with heterozygous *HIF2A* c.1121T>A mutation presented with a hemoglobin (Hb)/hematocrit (Hct)/Epo of 17.9 g/dL/54.4%/93.4 mIU/mL, diabetes mellitus and prior cerebrovascular accident (CVA)/ left ventricular thrombus, was started on phlebotomy, continued aspirin with anticoagulation and did not experience additional thromboses. The second case was a 56-year-old female with heterozygous *HIF2A* c.1121T>A mutation. Hb/Hct/Epo at presentation; 19.1 g/dL/57%/40.8 mIU/mL, with hypertension and hyperlipidemia, developed multiple thromboses; myocardial infarction, followed by CVA, inferior vena caval thrombus post-diagnosis, the latter occurred despite ongoing phlebotomy and aspirin/clopidogrel. The remainder two patients with heterozygous *HIF2A* c.1121T>A mutations were 68- and 71-year-old males with hypertension and hyperlipidemia respectively, Hb/Hct/Epo at diagnosis were 19.1/57.2/20.7 and 17.2/52/7.7, both did not experience thrombosis with the former receiving phlebotomy and the latter low dose aspirin.

Additionally, a 61-year-old female harbored a heterozygous missense alteration in *HIF2A* c.1620C>A, resulting in amino acid substitution p.Phe540Leu (F540L) previously reported by our group.[4] She had a history of hypertension, presented with Hb/Hct/Epo of 16.1/47.8/7.3 and did not experience thrombosis while on low-dose aspirin. On the other hand, a 69-year-old hypertensive male with heterozygous *HIF2A* c.1609G>A, mutation with Hb/Hct/Epo of 23/58.7/175 at diagnosis, developed a CVA with ongoing phlebotomy. An elevated Epo level (range, 20.7-175, reference range; 2.6-18.5 mIU/mL) was noted in four of six patients with *HIF2A* pathogenic variants, which in all instances was accompanied by phlebotomy. All patients had one or more cardiovascular risk factors, with three patients (50%) experiencing thrombosis, two of which occurred with ongoing phlebotomy, suggesting the lack of benefit of phlebotomy.

Of three patients with *PHD2* pathogenic variants; a 35-year-old female with family history of erythrocytosis, current smoker, without history of prior thrombosis, and Hb/Hct/Epo 17.2/52.6/11.2, demonstrated a *PHD2* c.1111C>T, p.(Arg371Cys) missense variant. This variant has been reported in the human gene mutation database,[6,7] and involves a highly conserved amino acid in the Fe(2+) 2-oxoglutarate dioxygenase domain, critical for hydroxylation of HIF; functional studies have not been performed but studies involving (Arg371His) have shown decreased ability of PHD2 to bind and hydroxylate HIF. On the other hand, two patients harbored previously reported *PHD2* c.461C>A, p.(S154*) and c.1030C>T, p.(Arg344*) nonsense variants predicted to result in a premature stop codon in exon 1 and 3, respectively, and expected to be loss of function mutations.[4] This included a 67-year-old male with *PHD2* c.461C>A and a 60-year-old female with *PHD2* c.1030C>T mutation, Hb/Hct/Epo at diagnosis were 17.8/50.7/10.3 and 17/not available/30, both did not experience thrombosis, former had known coronary artery disease and was on low-dose aspirin while the latter was hypertensive and receiving phlebotomy along with aspirin.

A pathogenic variant in *VHL* was detected in a 19-year-old male, compound heterozygous (L188V and R200W) for the previously described *VHL* mutations,[8] who presented with erythrocytosis (Hb/Hct 19/57) and a markedly elevated EPO level at 1465 mIU/mL. He was managed with phlebotomy every 4 weeks, in addition to aspirin and did not experience thrombosis.

Table 1. Clinical features and management of ten patients with *EGLN1(PHD2)/ EPAS1(HIF2A)/VHL* pathogenic variant associated erythrocytosis.

Patient n/ age at diagnosis/ sex	Gene mutation	Family history	Hb/HcT	EPO	p50	CV risks	Thrombosis (Therapy at event)	Pregnancy	Phlebotomy	Aspirin	Anticoagulation
#1 35/F	*EGLN1(PHD2)* Heterozygous c.1111C>T, p.(Arg371Cys)[6,7]	Sister	17.2/52.6	11.2		Smoking	none	2 live births	none	81 mg	none
#2 60/F	*EGLN1 (PHD2)* Heterozygous c.1030C>T, p.(Arg344*)[4]	none	17/	30	26	HTN	none	2 live births	HcT< 42	81 mg	none
#3 67/M	*EGLN1 (PHD2)* Heterozygous c.461C>A, p.(Ser154*)[4]	Brother x 2	17.8/50.7	10.3		CAD	none		none	81 mg	none
#4 69/M	*EPAS1 (HIF2A)* Heterozygous c.1609G>A, p.(Gly537Arg)[19]	none	23/58.7	175	27	HTN	CVA after diagnosis (phlebotomy)		Every 3 to 4 months HcT < 50	325 mg	none
#5 57/M	*EPAS1 (HIF2A)* Heterozygous c.1121T>A, p.(Phe374Tyr)[5]	none	17.9/54.4	93.4	27	DM	CVA prior to diagnosis LV thrombus (none)		HcT<45	325 mg	Enoxaparin apixaban
#6 56/F	*EPAS1(HIF2A)* Heterozygous c.1121T>A, p.(Phe374Tyr)[5]	none	19.1/57	40.8	26	HTN hyperlipidemia	MI CVA IVC thrombus after diagnosis (phlebotomy, aspirin, Plavix)		yes	Aspirin 81 mg Plavix 75 mg	warfarin
#7 68/M	*EPAS1(HIF2A)* Heterozygous c.1121T>A, p.(Phe374Tyr)[5]	none	19.1/57.2	20.7		HTN	none		HcT<50	none	none
#8 71/M	*EPAS1(HIF2A)* Heterozygous c.1121T>A, p.(Phe374Tyr)[5]	none	17.2/52	7.7		hyperlipidemia	none		none	81 mg	none
#9 61/F	*EPAS1(HIF2A)* Heterozygous c.1620C>A, p.(Phe540Leu)[20]	none	16.1/47.8	7.3	27	HTN	none		none	81 mg	none
#10 19/M	*VHL* Heterozygous 562C>G, p.(Leu188Val) c.598C>T, p.(Arg200Trp)[8]	none	19/57	1465	31	none	none		Every 4 weeks HcT <45	81 mg	none

Hb: hemoglobin; HcT: hematocrit; HTN: hypertension; DM: diabetes mellitus; CVA: cerebrovascular accident; LV: left ventricle; IVC: inferior vena cava; EPO: erythropoietin; p50: oxygen tension at which hemoglobin is 50% saturated.

Canonical exon 8 *EPOR* c.1316G>A mutations,[9] occurred in two patients, 48- and 69-year-old females, with a family history of erythrocytosis, and Hb/Hct/Epo levels of 19.4/56.6/1.1 and 14.6/44.3/<1, respectively, underscoring the suppressed Epo levels with gain of function *EPOR* mutations (Table 2). Both patients underwent intermittent phlebotomy and had an uncomplicated course in terms of thrombosis and pregnancies.

Two patients harbored *BPGM* pathogenic variants (Table 2) which included a 25-year-old male with hypertension who presented with Hb/Hct/Epo/p50 of 20/58/17.7/31, found to have a heterozygous missense alteration in *BPGM* at c.184C>T resulting in amino acid substitution p.Arg62Trp (R62W). While this specific amino acid change is novel, (p.Arg62Gln) has been reported in association with erythrocytosis in patients homozygous for the variant[10] and compound heterozygous for Arg62Gln and another *BPGM* pathogenic variant.[11] The second case was a 25-year-old male, current smoker with Hb/Hct/Epo of 17/49.1/5.1, who harbored a previously unreported *BPGM* c.258dup, p.(Leu87Serfs*3) frameshift variant in the first coding exon, predicted to result in a premature stop codon. Similar nonsense mutations leading to a predicted premature stop codon have been reported.[10,12,13] Both patients had an uneventful clinical course, the first patient was receiving phlebotomy and aspirin while the second case was observed.

Among 22 VUS that were reported, *PHD2* was most

Table 2. Clinical features and management of four patients with *EPOR/BPGM* pathogenic variant associated erythrocytosis.

Patient n/ age at diagnosis/ sex	Gene mutation	Family history	Hb/HcT	EPO	p50	CV risks	Thrombosis (therapy at event)	Pregnancy	Phlebotomy	Aspirin	Anticoagulation
#1 48/F	*EPOR* Heterozygous c.1316G>A, p.(Trp439*)[9]	Mother Brother	19.4/56.6	1.1		HTN	none	2 live births	Intermittent HcT< 50	none	none
#2 69/F	*EPOR* Heterozygous c.1316G>A, p.(Trp439*)[9]	Father Son Daughter	14.6/44.3	<1	27	none	none	2 live births	Every 4 to 8 weeks HcT< 43	81 mg	none
#3 25/M	*BPGM* Heterozygous c.184C>T, p.(Arg62Trp)[10,11]	Unknown	20/58	17.7	31	HTN	none		Every 6 weeks Hb< 14.5	81 mg	none
#4 25/M	*BPGM* Heterozygous c.258dup, p.(Leu87Serfs*3)[10,12,13]	none	17/49.1	5.1		Smoking	none		none	none	none

Hb: hemoglobin; HcT: hematocrit; HTN: hypertension; DM: diabetes mellitus; CVA: cerebrovascular accident; LV: left ventricle; IVC: inferior vena cava; EPO: erythropoietin; p50: oxygen tension at which hemoglobin is 50% saturated.

frequently involved (Table 3). The majority (n=17, 77%) of cases were males with median age at diagnosis of 50 years (range, 16-73 years). All patients had normal p50 testing, whereas EPO levels were highly variable, median 8 mIU/mL (range, 3.8-47.7 mIU/mL). A family history of erythrocytosis was known in five patients (23%) and thrombosis occurred in two (9%) of patients; the majority were managed with phlebotomy/blood donation (n=16, 73%) and/or antiplatelet therapy (n=12, 55%).

In the current series, we share our decades worth of hereditary erythrocytosis testing experience from the Mayo Clinic in order to define the incidence of alterations involving the hypoxia sensing pathway, in addition to *EPOR* and *BPGM*, providing a clinical perspective on the likelihood of encountering such abnormalities during the course of erythrocytosis evaluation. We limited the above series to the hypoxia sensing pathway genes, *EPOR*, and *BPGM*, since we have recently published on HOA variant associated erythrocytosis. Of the hypoxia sensing pathway alterations, homozygous *VHL* (598C>T) mutation Chuvash polycythemia [CP] is phenotypically well-characterized by an unusual propensity for vascular events leading to early mortality.[14] In a prospective, age, sex-matched controlled study on the subject matter, age and prior thrombotic events emerged as independent predictors of thrombosis; moreover, phlebotomy was associated with an increased incidence of thrombosis.[15] Similarly, among eight patients harboring the *HIF2A* p.M535V variant, five experienced thrombotic events *versus* none in 17 *HIF2A* wild-type patients.[15] Furthermore, thrombotic events occurred despite phlebotomy and in the absence of cardiovascular risks.[15] In our series, all three thrombotic events occurred in patients harboring *HIF2A* pathogenic variants, two of which were receiving phlebotomy, in addition to dual antiplatelet therapy in one patient. Of note, *HIF2A* alterations may be associated with neuroendocrine tumors such as pheochromocytoma, paraganglioma, somatostatinoma;[16] however, none of our patients with *HIF2A* alterations developed tumors. Limitations of our study

Table 3. Clinical features and management of 22 patients with variants of uncertain significance involving *EPOR/EGLN1(PHD2)/EPAS1(HIF2A)/VHL/BPGM* and associated erythrocytosis.

Variables	N=22
Gene, n	
EPOR	1
EGLN1 (PHD2)	10
EPAS1 (HIF2A)	3
VHL	6
BPGM	2
Age in years, median (range)	50 (16-73)
Male sex, n (%)	17 (77)
Hemoglobin g/dL, median (range)	18.2 (16-20.7)
Hematocrit %, median (range)	53.4 (48.5-60)
Serum erythropoietin mIU/mL, median (range) Reference range, 2.6-18.5 mIU/mL	8 (3.8-47.7)
p50 mm Hg, median (range)	n=19 25 (24-29)
Cardiovascular risk factors, n (%)	16 (73)
Family history, n (%)	5 (23)
Thrombosis[α], n (%)	2 (9)
Major arterial thrombosis	0
Major venous thrombosis	2
Treatment, n (%)	
Phlebotomy/blood donation	16 (73)
Antiplatelet therapy (aspirin or clopidogrel)	12 (55)
Anticoagulation	4 (18)

α. Major venous thrombosis included deep vein thrombosis, pulmonary embolism. *EPOR*- c.1310G>A, p.Arg437His, *EGLN1 (PHD2)*- c.826A>G, p.Met276Val, c.165G>C, p.Lys55Asn, c.709G>C, p.Asp237His, c.280A>G, p.Arg94Gly, c.1016G>C, p.Ser339Thr, c.112A>G, p.Ser38Gly , c.289G>A, p.Ala97Thr, c.586G>C, p.Glu196Gln, c.604A>G, p.Met202Val, c.*92G>A, single nucleotide substitution, *EPAS1 (HIF2A)*- c.1958C>T, p.Ala653Val, c.1556C>T, p.Thr519Met, c.1834G>A, p.Gly612Arg *VHL*- c.-61_-51het_dup11 (g.10183420), c.241C>T, p.P81S, c.134C>G ,p.45R, c.167C>G, p.Ala56Gly, c.345C>T, p.H155H, c.599G>A, p.Arg200Gln (heterozygous), *BPGM*- c.115C>T, p.Arg39Trp, c.289G>C, p.Gly97Arg. EPOR: erythropoietin receptor; *EGLN1* (PHD2): EGL-9 family hypoxia inducible factor 1 (prolyl hydroxylase 2); *EPAS1 (HIF2A)*: endothelial PASS domain protein 1(hypoxia-inducible factor 2); *VHL*: von Hippel Lindau; *BPGM*: 2,3-bisphosphoglycerate mutase.

include the retrospective design, and heterogeneity in clinical practice in regard to diagnosis and management.

In summary, we confirm the infrequent (0.5-2.2%) occurrence of genetic alterations involving the hypoxia pathway, *EPOR* and *BPGM* among patients undergoing hereditary erythrocytosis evaluation at the Mayo Clinic which includes testing for all congenital mutations except recently described *EPO* and iron-responsive element binding protein 1 (IRP1) mutations.[17,18] Additionally, phenotypic correlations and management details are provided which may serve as a useful guide for clinicians.

Naseema Gangat,[1] Jennifer L. Oliveira,[2] Tavanna R Porter,[2] James D. Hoyer,[2] Aref Al-Kali,[1] Mrinal M Patnaik,[1] Animesh Pardanani[1] and Ayalew Tefferi[1]

[1]*Division of Hematology, Department of Internal Medicine, Mayo Clinic, Rochester and* [2]*Division of Hematopathology, Department of Laboratory Medicine, Mayo Clinic, Rochester, MN, USA.*

Correspondence:

NASEEMA GANGAT - gangat.naseema@mayo.edu

AYALEW TEFFERI - tefferi.ayalew@mayo.edu

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