Germline mutations in the oxygen-sensing pathway (VHL-HIF2A-PH2D), or erythropoietin (EPO) signaling (EPO), 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-HIF2APH2D) or subnormal EPO (EPO). 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-bisphosphoglycerate mutase (BPGM) causing 2,3-BPG deficiency or methemoglobinemia. 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. 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. In a recent study which included 270 patients with idiopathic erythrocytosis, 1.1% harbored EPO mutations, while pathogenic variants involving genes in the hypoxia pathway were identified in 23% of patients. Accordingly, we share the Mayo Clinic clinical and laboratory experience with hereditary erythrocytosis resulting from genetic alterations in the oxygen-sensing pathway (VHL-HIF2A-PH2D), EPO 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 EPO (exon 8), hypoxia-inducible factor 2 alpha (HIF2A) encoded by endothelial PASS domain protein 1 (EPAS1) (exons 9 and 12), prolyl hydroxylase 2 (PH2D) encoded by EGL-N9 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. Of 592 patients tested at the Mayo Clinic for HIF2A/PHD2/EPO alterations, 14 pathogenic variants were identified in HIF2A (n=6, 1%), PH2D (n=3, 0.5%), EPO (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; EPO (n=1), HIF2A (n=3), PH2D (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 EPO, HIF2A, PH2D, BPGM; and VHL alterations, respectively.

Table 1 summarizes oxygen-sensing pathway (PH2D/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 homozygous HIF2A c.1121T>A, p.(Phe374Tyr) alteration in exon 9, previously reported in association with neuroendocrine tumors with or without erythrocytosis. 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 17.2/52.7/7.7, 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. 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 23/88/77 was managed by phlebotomy. The second case was a 56-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 PH2D c.1111C>T, p.(Arg371Cys) missense variant. This variant has been reported in the human gene mutation database, 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 PH2D to bind and hydroxylate HIF. On the other hand, two patients harbored previously reported PH2D 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. This included a 67-year-old male with PH2D c.461C>A and a 60-year-old female with PH2D c.1030C>T mutation, Hb/Hct/Epo at diagnosis were 17.8/50/7.10.3 and 17/40, 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, 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/ | Gene mutation | Family history | Hb/Hct | EPO | p50 | CV risks | Thrombosis (Therapy at event) | Pregnancy | Phlebotomy | Aspirin | Anticoagulation |
|-----------|---------------|----------------|--------|------|-----|----------|-----------------------------|------------|------------|---------|----------------|
| #1 35/F   | EGLN1(PHD2)   | Sister         | 17.2/52.6 | 11.2 | Smoking | none | 2 live births | none | 81 mg | none |
| #2 60/F   | EGLN1(PHD2)   | none           | 17/     | 30   | 26   | HTN      | none | 2 live births | Hct<42 | 81 mg | none |
| #3 67/M   | EGLN1(PHD2)   | Brother x 2    | 17.8/50.7 | 10.3 | CAD  | none | none | 81 mg | none |
| #4 69/M   | EPAS1(HIF2A)  | none           | 23/58.7 | 175  | 27   | HTN      | CVA after diagnosis | (phlebotomy) | 325 mg | none |
| #5 57/M   | EPAS1(HIF2A)  | none           | 17.9/54.4 | 93.4 | 27   | DM       | CVA prior to diagnosis | LV thrombus | (none) | 325 mg | Enoxaparin apixaban |
| #6 56/F   | EPAS1(HIF2A)  | none           | 19.1/57 | 40.8 | 26   | HTN      | MI CVA | IVC thrombus | after diagnosis | (phlebotomy, aspirin, Plavix) | yes | Aspirin 81 mg Plavix 75 mg | warfarin |
| #7 68/M   | EPAS1(HIF2A)  | none           | 19.1/57.2 | 20.7 | HTN  | none | Hct<50 | none | 81 mg | none |
| #8 71/M   | EPAS1(HIF2A)  | none           | 17.2/52 | 7.7  | hyperlipidemia | none | none | 81 mg | none |
| #9 61/F   | EPAS1(HIF2A)  | none           | 16.1/47.8 | 7.3  | 27   | HTN      | none | none | 81 mg | none |
| #10 19/M  | VHL           | 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, 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 14.6/44.3/3.1, 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 and compound heterozygous for Arg62Gln and another BPGM pathogenic variant. 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. 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
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 EPO 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, EPO, 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,6 however, none of our patients with HIF2A alterations developed tumors. Limitations of our study

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

| Patient # | Age at diagnosis | Gender | Erythrocytosis genotype | Hypoxia gene alteration | Family history | History of CV risks | Pregnancy | Phlebotomy | Aspirin | Anticoagulation |
|-----------|------------------|--------|-------------------------|-------------------------|----------------|---------------------|-----------|------------|---------|---------------|
| #1        | 48/F             | Heterozygous | c.1316G>A, p.(Trp439*) | c.599A>G, p.(Arg200Gln) | Mother Brother | HTN                 | Intermittent Hp<50 | none       | none     | none         |
| #2        | 50/F             | Heterozygous | c.1316G>A, p.(Trp439*) | c.599A>G, p.(Arg200Gln) | Father Son Daughter | none | 2 live births | Every 4 to 8 weeks Hp<50 | 81 mg | none |
| #3        | 25/M             | Heterozygous | c.1316G>A, p.(Trp439*) | c.258dup, p.(Leu97Serfs*1) | Unknown | 17/49.1 | 5.1 | Smoking | none | none | none |
| #4        | 25/M             | Heterozygous | c.1316G>A, p.(Trp439*) | c.599A>G, p.(Arg200Gln) | 17/49.1 | 5.1 | Smoking | none | none | none | none |

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

| Variables                                      | N=22 |
|------------------------------------------------|------|
| Gene, n                                        |      |
| EPOR                                           | 1    |
| EPAS1 (HIF2A)                                  | 5    |
| 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 (48.3-82) |
| Serum erythropoietin mIU/mL, median (range)    | 8 (3.8-47.7) |
| Reference range, 2.6-18.5 mIU/mL               |      |
| p50 mm Hg, median (range)                      | 19 (25-24.9) |
| Cardiovascular risk factors, n (%)             | 16 (73) |
| Family history, n (%)                          | 5 (23) |
| Major thrombosis                               | 2 (9) |
| Major cardiac event                            | 0 |
| Major venous thrombosis                        | 2 |
| Treatment, n (%)                               |      |
| Phlebotomy/blood donation                      | 16 (73) |
| Antiplatelet therapy (aspirin or clopidogrel)  | 12 (55) |
| Anticoagulation                                | 4 (18) |

a. Major venous thrombosis included deep vein thrombosis, pulmonary embolism.

**EPOR:** c.1316G>A, p.Arg439Ter; **EPAS1 (HIF2A):** c.826A>G, p.Arg276Gly; c.1650C>G, p.Lys550Arg; c.790G>C, p.Arg267Trp; c.2860G>A, p.Arg953Gly; c.1016G>C, p.Ser339Gly; c.1124G>T, p.Ser375Leu; c.357G>A, p.Glu119Glu; c.1635C>T, p.Glu545Glu; c.1148C>T, p.Glu383Glu; p.Leu97Stop, p.1155C>T, p.H1155Lys, p.599G>A, p.Arg200Gln (heterozygous); **BPGM:** c.345C>T, p.H1155Lys, p.599G>A, p.Arg200Gln (heterozygous); **VHL:** c.345C>T, p.H1155Lys, p.599G>A, p.Arg200Gln (heterozygous).
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, EPO 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 (IRE1) mutations. Additionally, phenotypic correlations and management details are provided which may serve as a useful guide for clinicians.

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