Congenital polycythemias/erythrocytoses

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Congenital polycythemias may result from inherited defects in hypoxia sensing, from inherited intrinsic defects in red blood cell precursors, or from inherited conditions that cause low tissue oxygen tension and secondary polycythemia. Conditions of defective hypoxia sensing feature inappropriately normal or elevated serum erythropoietin (Epo) concentrations in the setting of normoxia and erythrocytosis. They are often due to homozygous or compound heterozygous germline mutations in the von Hippel-Lindau tumor suppressor gene (*VHL*) but without increased incidence of tumors. Affected persons have a high risk of arterial thrombosis and early mortality. The molecular biology of rare polycythemic patients with a single mutated *VHL* allele remains obscure. Primary congenital and familial polycythemias are characterized by low Epo levels and increased erythroid precursor responsiveness to Epo. They are often due to heterozygous gain-of function mutations in the gene for erythropoietin receptor (*EPOR*). Secondary congenital polycythemias have low tissue oxygen tension due to hemoglobins with high affinity for oxygen, low erythrocyte 2,3 biphosphoglycerate levels, methemoglobinemia or cyanotic heart or lung disease. Whether phlebotomy therapy reduces complications and prolongs survival in congenital polycythemia is not known.

Key words: polycythemia, von Hippel-Landau gene, ataxia-telangectasia.

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The Greek term, polycythemia, is synonymous with the word erythrocytosis, and literally translates as many cells in the blood. There are multiple causes of polycythemia, and these can be conveniently classified broadly as acquired and congenital disorders (Table 1). Polycythemias can also be classified as primary or secondary disorders. Primary polycythemias result from abnormalities expressed within hematopoietic progenitors, while secondary polycythemias are due to circulating factors that act on these progenitors, in most instances erythropoietin (Epo). In other words, in primary polycythemias there is an innate defect in the hematopoietic progenitors which allows constitutive overproduction whereas in secondary polycythemias normal progenitors are acted on by external factors. Both acquired and congenital polycythemias can be primary or secondary. Polycythemia vera, an acquired condition characterized by clonal expansion of hematopoietic precursors, is the most common primary polycythemia.[1] Rarely, polycythemia vera occurs in several members of the same family wherein it is typically acquired and the inherited risk is not fully penetrant.[2] Acquired conditions that lead to increased Epo production, such as chronic hypoxia and a variety of tumors, are the most common causes of secondary polycythemias. These important disorders are not the subject of this review and will not be discussed further.

Congenital polycythemia can be primary and result from inherited defects in hypoxia sensing or from inherited intrinsic defects in red blood cell precursors that cause increased responsiveness to Epo (primary familial and congenital poly-

Table 1. Classification of polycythemias.

Disorder	*Mathematical presentation of Epo level and reference*	*Serum Epo level non-phlebotomized (IU/L)*	*Serum Epo level phlebotomized (IU/L)*
Acquired polycythemias			
Polycythemia vera	Mean (SD)[44]	0.5 (0.9)	2.5 (2.9)
Polycythemia secondary to increased Epo secretion			
Chronic hypoxia due to lung disease	NA	Normal to Increased	Increased
Tumors that secrete Epo	NA	Increased	Increased
Post-renal transplant	Mean (SD)[45]	9.6 (9.6)	
Polycythemia secondary to other factors			
Cobalt exposure	Insufficient data	Insufficient data	Insufficient data
Increased IGF1 levels	Insufficient data	Insufficient data	Insufficient data
Congenital polycythemias			
Congenital polycythemias due to oxygen sensing defect			
Homozygous Chuvash VHL mutation	Median (range)[11]	20 (12-69)	75 (25->200)
Other *VHL* genotypes	Median (range)[17-19]	43 (26-271)	Increased
Mutation not identified		Normal to Increased	
Primary familial and congenital polycythemias			
EPOR mutation	Median (range)[30,46,47]	4 (<10)	17
Mutation not identified			
Disorders of altered affinity of hemoglobin for O_2			
Hemoglobins with increased O_2 affinity		Normal to Increased	Increased
2,3 BPG deficiency		Normal to Increased	Increased
Methemoglobinemia		Normal to Increased	Increased
Congenital cyanotic heart disease	Geometric mean (95% range)[48]	9 (3-26)	Increased

NA: not available; When numerical values are not reliable, the magnitude is estimated as normal, increased normal to increased, etc.

cythemia). Congenital polycythemia can also be secondary to inherited conditions that lead to increased Epo levels. Examples include hemoglobins with high affinity for oxygen, low erythrocyte[2,3] biphosphoglycerate levels, and congenital cyanotic heart or lung disease.

Congenital polycythemia due to altered hypoxia sensing

Regulation of oxygen homeostasis is critical to survival. Hypoxia results in increased levels of hypoxia-inducible factor (HIF1), which is part of a widespread O_2-sensing mechanism providing transcriptional regulation of *EPO* and many other hypoxia-regulated genes.[3] HIF1 is composed of two subunits, HIF1α and HIF1β, which form a heterodimer;[4] only HIF1α is regulated by hypoxia. Normoxia-induced ubiquitin-mediated degradation of HIF1α protein is the major regulator of HIF1α levels.[5] Cellular HIF1α protein levels are increased by hypoxia and HIF1α protein decays rapidly with return to normoxia.[6] The targeting and subsequent polyubiquitination of HIF1α requires von Hippel Lindau protein (pVHL), iron, O_2 and a unique proline hydroxylase activity; this complex constitutes the oxygen sensor.[7,8]

Chuvash polycythemia – homozygous VHL mutation

Chuvash polycythemia (CP) is the only known endemic congenital polycythemia. Our recent studies indicate that this form of polycythemia is due to an abnormality in the oxygen-sensing pathway. A Russian hematologist, Dr. Lydia A Polyakova,[9] first described Chuvash polycythemia, which is common in the Chuvash population of Russia. It is estimated that there may be hundreds of affected individuals among the two million people of this ethnic group of Central Asian descent. CP is associated with early mortality due in part to arterial thrombosis.[9-11]

The Chuvash people are one of the central Asian Bulgar tribes who migrated northward to the mid-Volga River region about 1000 years ago. The Chuvash, who converted to Orthodox Christianity, tended to be culturally as well as geographically isolated from the surrounding tribes, who remained Muslim. Thus, the Chuvash population appears to be fairly homogeneous in ethnicity, and the presence of this hereditary polycythemic condition appears to represent a founder effect. Exploiting this founder effect in a study of five multiplex Chuvash families with CP, we used a genome-wide screen to localize the CP region on chromosome 3 with a LOD score of over 3.5. After sequencing several candidate genes, we identified a C to T transition at nucleotide 598 (an

Table 2. Chuvash polycythemia: clinical and laboratory findings by *VHL* genotype of matched cohort study participants who were alive in 2001.

	VHL 598C→T homozygote (n=43)	*VHL 598C→T heterozygote (n=9)*	*Wildtype VHL (n=77)*	*p**
History and physical examination				
Age (years; mean ± SE)	49±1	45±3	50±2	0.7
Female sex (no. (%)	32 (74.4)	6 (66.7)	38 (49.4)	0.008
Thrombosis by history (no. (%)	11 (25.6)	0 (0)	1 (1.3)	<0.0005
Cancer by history (no. (%))	0 (0)	0 (0)	1 (1.3)	0.5
Body mass index (kg/m^2; mean±SE)	23.2±0.6	27.0±1.4	24.9±0.5	0.038
Systolic BP (mm Hg; mean±SE	120±3	119±6	133±2	0.001
Diastolic BP (mm Hg; mean±SE)	79±2	78±5	87±2	0.002
Varicose veins by PE [no. (%)]	32 (74.4)	2 (22.2)	30 (39.0)	<0.0005
Laboratory values				
Hemoglobin (g/dL; mean±SE)	18.3±0.3	13.3±0.4	12.9±0.2	<0.0005
Serum ferritin (μg/L; geometric mean & SE range)	67 (37-122)	20 (12-34)	30 (22-41)	0.3
Epo (IU/L; geometric mean [SE range])	76 (67-86)	8 (6-9)	7 (7-8)	<0.0005
Transferrin receptor (mg/L; geo. mean [SE range])	14.2 (13.0-15.5)	4.8 (4.0-5.7)	5.7 (5.3-6.0)	<0.0005
Serum PAI-1 (ng/mL; mean±SE)	110±3	96±7	82±2	<0.0005
Serum VEGF (pg/mL; geometric mean [SE range])	109 (102-117)	84 (72-97)	74 (70-78)	<0.0005

**p value for comparison between VHL 598C>T homozygotes and VHL wildtype participants by analysis of variance or by Fisher's exact test. Blood pressures and PAI-1 levels also differed significantly between heterozygotes and unaffected participants (p< 0.05). Blood pressure adjusted for body mass index; hemoglobin for sex; ferritin for age, sex and phlebotomy; transferrin receptor for ferritin; PAI-1 and VEGF for platelet count.*

R200W mutation) in the von Hippel-Lindau (*VHL*) gene. All of the patients with CP were homozygotes for this mutation, while all obligate carriers were heterozygotes.[12]

Molecular studies in patients with CP indicate that *VHL 598C→T* leads to impairment of the interaction of pVHL with HIF1α, reducing the rate of ubiquitin-mediated degradation of HIF1α and resulting in increased levels of the active dimer HIF1 and expression of downstream target genes including *EPO*, *SLC2A1* (also known as *GLUT*1, encoding facilitated glucose transporter member 1 of solute carrier family 2), transferrin (*TF*), transferrin receptor (*TFRC*, encoding p90, CD71) and vascular endothelial growth factor (*VEGF*).[13]

Because CP is characterized by a germline mutation in the *VHL* gene, we hypothesized that homozygotes for this mutation may develop certain vascular tumors similar to those associated with the classic VHL syndrome. In a matched cohort study, *VHL598C→T* homozygosity was associated with varicose veins, lower blood pressures and elevated serum VEGF and PAI-1 concentrations (p<0.0005), as well as premature mortality related to cerebral vascular events and peripheral thrombosis. Tumors typical of the classic VHL syndrome, such as spinocerebellar hemangioblastomas, renal carcinomas and pheochromocytomas, were not found, indicating that increased expression of HIF1α and VEGF is not sufficient for tumorigenesis.[11] In this investigation, we studied 96 patients with CP diagnosed before 1977, 65 spouses, and 79 community members of the same age, sex, and village of birth. Estimated survival to 65 years was ≤31% for CP patients versus ≥67% for spouses and community members (p≤0.002; Figure 1). We were able to obtain blood samples from 43 patients and 86 spouses or community members who were living at the time of the study, and found a perfect genotype-phenotype correlation for clinically diagnosed CP with all patients, but no others, genotyped as homozygotes for the *VHL598C→T* missense mutation.[12,13] CP was associated with high serum total plasminogen activator inhibitor-1 (PAI-1, a HIF1 regulated gene) levels and a history of thrombosis, as well as with high serum VEGF levels, relatively low blood pressures and varicose veins (Table 2). Imaging studies in 33 *VHL598C→T* homozygotes revealed unsuspected cerebral ischemic lesions in 45% but no spinocerebellar hemangioblastomas, renal carcinomas, or pheochromocytomas. Benign vertebral body hemangiomas (a distinct entity from hemangioblastoma) were found in 55% of patients with CP versus 21% of control Chuvash patients without polycythemia (p=0.006). Hemoglobin-adjusted serum Epo concentrations were approximately 10-fold higher in *VHL598C→T* homozygotes than in controls (Table 2), but, as shown in Figure 2, the Epo response to hypoxia was identical. Thus, CP is a distinct VHL syndrome manifested by thrombosis, vascular abnormalities and intact hypoxic regulation despite increased basal expression of hypoxia-regulated genes. It is characterized by increased systemic expression in normoxia of a broad range of HIF1-regulated genes and little or no predisposition

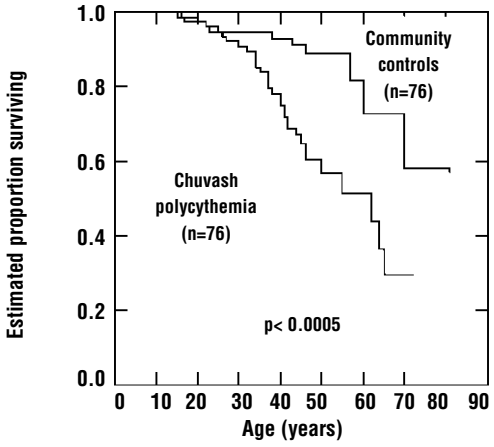

Figure 1. Kaplan-Meier survival curves for 76 Chuvash polycythemia patients and 76 community members who survived to 16 years, matched for age, sex and place of birth.

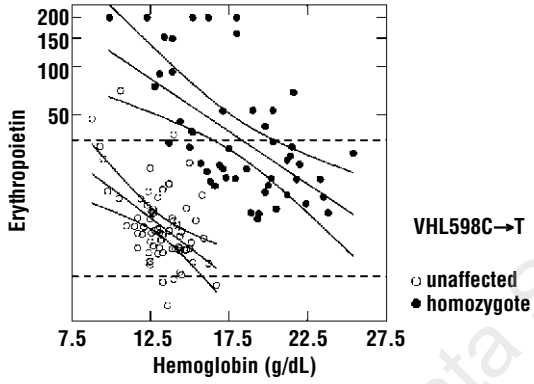

Figure 2. Response to hypoxia is intact in Chuvash polycythemia despite increased basal expression of hypoxia-regulated genes. The relationship of serum Epo concentration to hemoglobin concentration in 41 VHL 598C→T homozygotes and 70 unaffected participants, all of Chuvash ethnicity is depicted. Regression lines and 95% confidence intervals are shown for each group. The dashed horizontal lines represent the lower (5 IU/L) and upper (35 IU/L) limits of the reference range for Epo. The slope of the regression line is identical for each group (-0.007 log Epo unit per 1 g/dL hemoglobin).

to develop malignancy. Erythroid progenitors of CP patients are hypersensitive to Epo, thus CP shares features of both primary and secondary polycythemias. The molecular mechanism of this erythroid hypersensitivity remains to be elucidated. One possible explanation is that erythroid progenitors synthesize Epo in an autocrine fashion[14] and this erythroid Epo may be necessary for terminal differentiation and hemoglobinization.[15] If the erythroid Epo is upregulated this may explain the augmented erythropoiesis. Another possible mechanism, proposed on the basis of the observation that, while iron markedly increases the number of surviving erythroid progenitors in HIF1 wildtype embryos, HIF1-α knockout embryos have defective erythropoiesis that can be only partially improved by delivering iron to the cells. This finding suggests the existence of a HIF1-regulated iron-containing/dependent substance that promotes erythropoiesis.[16]

Table 3. *VHL* mutations associated with congenital polycythemia.

VHL Genotype	*Ethnicities*	*References*	
235 C→T/586 C→G	Caucasian	21	Frequent thrombotic complications
598 C→T/598 C→T	Chuvash, Bangladesh, Danish, Pakistani, Russian, US (white)	12,13,17-21	
598 C→T/574 C→T	US (white)	19	
598 C→T/562 C→G	US (white)	19	
598 C→T/388 G→C	US (white)	17	
571 C→G/571 C→G	Croatian	19	
311 G→T/ wildtype	German (?)	20	
376 G→T/ wildtype	Ukranian	Pastore *et al.*, 2003a	*VHL* syndrome
598 C→T/ wildtype	English, German	20	
523 A→G/ wildtype	Portuguese	21	A-T patient

A-T: ataxia-telangiectasia.

Other congenital polycythemias characterized by VHL mutations

Rapidly accumulating data indicate that other congenital polycythemias around the world are also due to defects in the oxygen sensing pathway and many of them are caused by mutations in *VHL*. Our recent studies of Chuvash polycythemia prompted us and others to examine a potential role for *VHL* mutations in patients with congenital polycythemias with high or inappropriate Epo levels for the level of hematocrit from other parts of the world than Chuvashia (Table 3).

We found homozygosity for *VHL598C→T*, the Chuvash mutation, in two Danish siblings and an American boy of Caucasian descent with congenital polycythemia. In addition, a polycythemic American girl adopted from Russia was homozygous for *VHL 598C→T*.[17] Other investigators have found homozygosity for *VHL598C→T* in polycythemic family members of Punjabi/Bangladeshi Asian ancestry.[18] Some patients with congenital polycythemia have proven to be compound heterozygotes for the Chuvash mutation and other *VHL* mutations.[17,19] Two unrelated Americans of Caucasian descent were compound heterozygotes for *VHL598C→T* and *562C→G* and a third was a compound heterozygote for *VHL598C→T* and *574C→T*. A boy of Italian, Dutch, German, Irish, and American Indian ancestry was a compound heterozygote for *VHL598C→T* and *388C→G*. Additionally, a Croatian boy was homozygous for *VHL571C→G* mutation, the first example of a homozygous *VHL* germline mutation, other than

the *VHL598C→T* mutation, causing polycythemia. Ten more polycythemic patients with *VHL* mutations are reported in this issue by Holfger Cario *et al*.,[20] and Celeste Bento *et al*.[21]

A few cases of congenital polycythemia that appear to have mutations of only one *VHL* allele confound an obvious pathophysiological explanation. In a Ukrainian family, two children with polycythemia were heterozygotes for *VHL3768G→T* (D126Y) but their father with the same mutation was not polycythemic.[17] One of the children had a pulmonary angioma and several years later developed a renal subcapsular hemangioma. Peripheral blood erythroid progenitors from the children and father were hypersensitive to recombinant Epo in *in vitro* clonogenic assays in a way similar to what is seen in CP patients. The propositus' peripheral granulocytes and platelets were polyclonal as determined by an X-chromosome based transcriptional clonality assay for which she was polymorphic,[22] arguing against an additional somatic mutation of a hematopoietic progenitor leading to clonal hematopoiesis akin to polycythemia vera.

However, one may argue that this individual may have been polycythemic as a result of an as yet unrecognized Epo-secreting tumor since the propositus had some clinical characteristics of *VHL* tumor predisposition syndrome, and some *VHL* tumors secrete Epo. An English patient was a heterozygote for *VHL598C→T*;[18] however, the inheritance of deletion of a *VHL* allele in a trans position was not excluded. There are two reports in this issue by Holger Cario *et al*.,[20] and Celeste Bento *et al*.[21] describing two separate *VHL* heterozygous patients in whom a null allele was more rigorously excluded; the molecular mechanism of their polycythemic phenotype remains to be elucidated.

We have not observed *VHL* syndrome-associated tumors in these polycythemic subjects or their heterozygous relatives; however, this aspect will need to be evaluated by longitudinal studies. Overall, we found that almost one-half of consecutive patients with apparent congenital polycythemia and increased serum Epo have mutations of both *VHL* alleles. These findings, along with reports of Chuvash polycythemia, underscore that *VHL* mutations are, thus far, the most frequent cause of congenital polycythemia and define a new class of polycythemic disorder, polycythemias due to dysregulated hypoxia sensing. Based on these data, we conclude that inheritance of mutations in the *VHL* gene is a newly described cause of congenital polycythemia and needs to be considered, particularly in those with an increased or inappropriately normal serum Epo concentration for the elevated hematocrit level. Surprisingly, inheritance of *VHL* mutations in both alleles (homozygosity and compound heterozygosity) is compatible with life and therefore implies a mild *VHL* functional defect.

A common ancestor for the Chuvash polycythemia mutation in diverse ethnic groups

The *VHL598C→T* (Chuvash) mutation has been identified in homozygous or heterozygous form in persons of Chuvash, white American, Danish, Asian, and African-American ancestry. To address the question of whether the *VHL598C→T* substitution occurred in a single founder or resulted from recurrent mutational events, haplotype analysis of eight highly informative single nucleotide polymorphic markers covering 340 kb spanning the *VHL* gene was performed on 101 subjects bearing the *VHL598C→T* mutation and 447 normal unrelated individuals from Chuvash, South-East Asian, Caucasian, Hispanic and African-American ethnic groups.[23] The differences in allele frequencies for each marker between 447 normal controls (598C) and 101 subjects bearing 598T were highly significant ($p<10^{-7}$), indicating strong linkage disequilibrium. Thus, we estimate that the *VHL598C→T* mutation arose in a single ancestor between 12,000 and 51,000 years ago.

It is possible that this wide dissemination from the original founder may be associated with some survival advantages for heterozygotes carrying this mutation. Such an advantage might be related to a subtle improvement of iron metabolism, erythropoiesis, embryonic development, energy metabolism[3] or some other yet unknown effect. An intriguing possibility is raised by the recent demonstration of a protective role for HIF-1α in regulating VEGF in pre-eclampsia,[24,25] the leading cause of maternal and fetal mortality worldwide.[26] Another potential protective role of a mildly augmented hypoxic response is improved protection against bacterial infections, since the hypoxia-mediated response was recently reported to be essential for the bactericidal action of neutrophils.[27] Four other Caucasian patients described in this issue[20,21] had the *VHL598C→T* mutation on the previously reported ancient haplotype. However, in this issue, Holger Cario and his colleagues[20] report a patient of Turkish ancestry who was homozygous for the *Chuvash polycythemic VHL 598C→T* mutation but this mutation was present on a completely different haplotype than that in all previous patients bearing the CP mutation, indicating that it likely represents an independent mutational event.

Congenital polycythemia due to altered oxygen sensing but without mutation of VHL

More than one-half of patients with congenital polycythemias with normal or elevated Epo levels do not have *VHL* mutations, and the molecular basis of those people's disease remains to be elucidated. Lesions in genes linked to oxygen-dependent gene regulation and their interacting proteins are leading

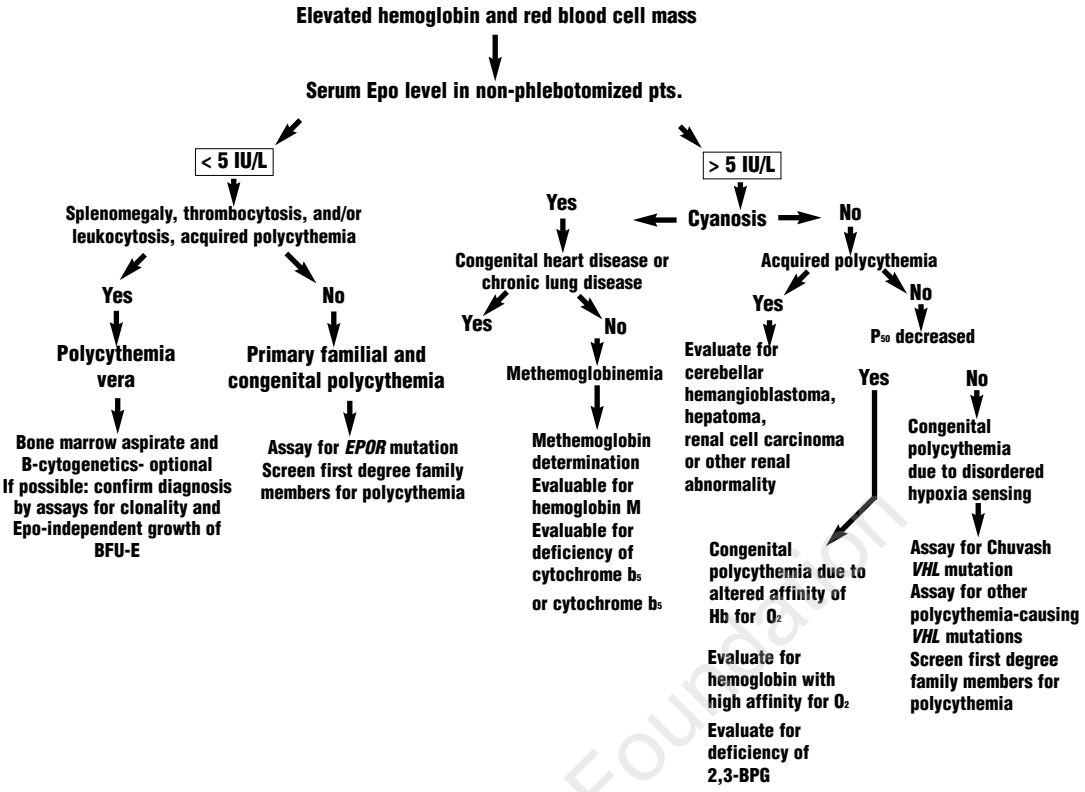

Figure 3. A diagnostic algorithm for polycythemia based on serum Epo concentration. Bx – biopsy, Epo IU/L – concentration of erythropoietin expressed in International units per liter.

candidates for mutation screening in polycythemic patients with normal or elevated Epo without *VHL* mutations. Some of these are inherited in a dominant fashion.[28]

Primary familial and congenital polycythemia

Primary familial and congenital polycythemia (PFCP) is to be contrasted with Chuvash polycythemia because the inheritance of PFCP is autosomal dominant rather than autosomal recessive,[29-33] and the condition is primary (i.e. defect in the erythroid progenitor and low Epo levels) rather than secondary to altered hypoxia sensing and a related increase in Epo secretion. Although PFCP is uncommon, it is more prevalent than polycythemias due to high oxygen affinity hemoglobin mutants or 2,3-biphosphoglycerate deficiency.[33] PFCP patients do not have splenomegaly and the disease does not progress to leukemia. Although generally felt to be benign, it is possible that this condition predisposes patients to severe cardiovascular problems.[34,35] An increased incidence of cardiovascular disease was observed in affected members of PFCP families.[36] Characteristic laboratory findings are: (i) an increased red blood cell mass without increases in leukocyte or platelet counts; (ii) normal vitamin B12 levels; (iii) normal hemoglobin-oxygen dissociation; (iv) low serum Epo levels; and (v) *in vitro* hypersensitivity of erythroid progenitors to Epo.[30,31] In searching for the molecular lesion resulting in the PFCP phenotype, mutations of *EPO* or its receptor, *EPOR*, were likely candidates.[32] We first excluded *EPO* mutations.[32] Next, cloning of *EPOR*[37,38] enabled this gene to be analyzed for mutations in subjects with PFCP. To date, 12 mutations of *EPOR* have been described. Nine out of the 12 result in truncation of the EpoR cytoplasmic carboxyl terminal and are the only mutations convincingly associated with PFCP.[33] Such truncations lead to a loss in the negative regulator domain of the EpoR, associated with SHP-1, and reinforce the crucial importance of retained positive regulatory domains associated with the JAK2/STAT5 proteins. Three missense *EPOR* mutations have been described, but these have not been linked to PFCP or any other disease phenotype. We have created a mouse model of PFCP based on a human disease-causing mutation that produces truncation of EpoR.[39] However, a different mouse model of truncated

EpoR (which was randomly created and not isolated from a PFCP subject) did not have a polycythemic phenotype.[40] The effect of a truncated EpoR in the host milieu is not always predictable. Some patients who inherit an *EPOR* mutation are not polycythemic, indicating that gene modifiers or epigenetic factors may mask the full PFCP phenotype. In a recent study, mutations of the *EPOR* were found in only 12% of subjects with PFCP, suggesting that in a majority of PFCP families, mutations in genes other than *EPOR* result in defective Epo signaling and accumulation of erythrocytes.[41,42]

Other causes of congenital polycythemia

Cyanotic congenital heart disease is an important cause of polycythemia in young children worldwide. Inherited conditions that increase the affinity of hemoglobin for oxygen are important but rare causes of congenital polycythemia. These conditions include high affinity hemoglobin disorders, deficiency of 2,3 BPG, and methemoglobinemia due to hemoglobin M or to deficiency of cytochrome b5 reductase.

Approach to the diagnosis of congenital polycythemia

A diagnostic algorithm for polycythemia in general, based on serum Epo concentration in non-phlebotomized patients, is presented in Figure 3. The advent of highly sensitive assays makes it possible to classify primary polycythemias as those in which serum Epo levels are below the normal range. Physical examination, routine laboratory tests, bone marrow examination, cytogenetics, and assays for clonality and Epo-independent growth of erythroid progenitors help to distinguish between acquired polycythemia vera and congenital PFCP as the cause of primary polycythemia. In polycythemic conditions with a serum Epo level in the normal range or above it, the presence of cyanosis on physical examination suggests chronic lung disease, congenital heart disease, or methemoglobinemia. In patients without cyanosis, determining the hemoglobin oxygen dissociation P_{50} will help to distinguish between conditions that increase the affinity of hemoglobin for oxygen and those that are likely characterized by disordered hypoxia sensing. Since equipment for measuring hemoglobin oxygen dissociation is no longer widely available, there is a simple formula that permits the value of P_{50} to be estimated from venous blood gases.[43]

VRG: developed the outline for the paper and drafted the Tables and Figures; DVS: participated in and supervised generation of the novel genetic information and participated in writing the manuscript and corrected the genetic nomenclature used; JTP: generated most of the molecular data in his laboratory and conceptualized the paper and classification of polycythemic disorders.

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