CHAPTER 8

Familial and acquired polycythaemia

8.1 Familial polycythaemia

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1. Introduction
Our understanding of the regulation of erythropoiesis has improved in recent years. The elucidation of the pathophysiology of congenital polycythaemias (erythrocytoses) provides new information regarding the molecular control of red cell production and leads to precise diagnosis. In this chapter, we review the pathophysiology of erythropoiesis and those clinical congenital disorders wherein the molecular basis has been defined. These can be divided into three categories.
1. Familial polycythaemia may be due to an intrinsic red cell precursor defect that leads to increased erythropoiesis despite low erythropoietin (Epo) levels. This is called primary familial polycythaemia and is often due to a gain-of-function mutation in the erythropoietin receptor gene.
2. Familial polycythaemia may be due to inherited defects that cause tissue hypoxia and a secondary increase in erythropoietin concentrations. Examples include haemoglobins with high oxygen affinity, defects that lead to methaemoglobinaemia or deficiency of 2,3-BPG.
3. Familial polycythaemia may be due to inherited defects that lead to increased erythropoietin concentrations in the setting of normal tissue oxygenation. These conditions of abnormal hypoxia sensing may result from mutations in the von Hippel-Lindau (VHL) gene, the gene for proline hydroxylase domain protein 2 or the gene for hypoxia inducible factor-2α.
Once thought to be benign, it is now clear that many forms of familial polycythaemia are complicated by thrombotic and other non-erythroid manifestations and increased mortality. Optimal therapy has not been determined. While polycythaemia vera (PV) is the most frequent primary polycythaemic disorder and is acquired, about 5% of PV patients have relatives with PV or with other myeloproliferative disorders. This suggests that these families inherit a yet-to-be identified gene(s) that increases the acquisition of PV somatic mutation(s).

2. Regulation of erythropoiesis as it relates to familial polycythaemia

2.1 Stages of erythropoiesis
The production of erythrocytes, erythropoiesis, is a tightly regulated process, but all aspects of its regulation are not fully elucidated. Much remains to be learned from uncovering the molecular basis of congenital and acquired mutations that disrupt the control of erythropoiesis. The process of erythropoiesis can be viewed as having three stages. In the initial stage, pluripotent haematopoietic progenitors transform to committed erythroid precursors. In the second stage, erythroid progenitors proliferate by a process that is largely regulated by Epo and is made effective by the appearance of its receptor (EpoR). Expression of EpoR peaks on the
surface of early erythroblasts during this stage and then declines. In the third or terminal stage, late erythroid precursors undergo enucleation and removal of nucleotides and other remnants of organelles that may be toxic to mature circulating erythrocytes. Defects in erythropoiesis that lead to polycythaemia invariably occur at the second stage of erythropoiesis.

2.2 Alterations in regulation that lead to polycythaemia

In this review we will concentrate only on those defects that have been demonstrated to lead to, or potentially could lead to, polycythaemia, i.e. increased red cell mass. Polycythaemia is synonymous with the term “erythrocytosis”, and since no consensus on usage has been reached, we will refer to individual entities by the conventional usage associated with them. Polycythaemias can be classified as being appropriate or inappropriate (Table 1A).

| Table 1: Classification of polycythaemia by two approaches |
|---------------------------------------------|---------------------------------------------|
| **A. Classification according to appropriateness (tissue oxygenation)** | **Acquired** |
| Congenital | Acquired |
| Appropriate polycythaemias (tissue hypoxia present) | Cardiac and pulmonary disorders characterised by hypoxia |
| Haemoglobins with high affinity for oxygen | Exposure to cobalt |
| Methaemoglobinaemia | Smoking and increased carboxyhaemoglobin |
| Low erythrocyte 2,3-biphosphoglycerate levels | |
| Inappropriate polycythaemias (tissue hypoxia absent) | |
| Primary and familial congenital polycythaemia | Polycythaemia vera |
| Congenital polycythaemias due to abnormal hypoxia sensing | Ectopic secretion of erythropoietin by tumours |

<table>
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<th><strong>B. Classification according to whether or not erythropoietin is increased</strong></th>
<th><strong>Acquired</strong></th>
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<tr>
<td>Congenital</td>
<td>Acquired</td>
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<tr>
<td>Primary (erythropoietin not increased; defect intrinsic to erythroid progenitor cells)</td>
<td>Polycythaemia vera</td>
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<tr>
<td>Secondary (erythropoietin increased)</td>
<td>Acquired cardiac and pulmonary disorders characterised by hypoxia</td>
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<tr>
<td>Congenital heart disease with right to left shunting</td>
<td>Exposure to cobalt or cyanate</td>
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<tr>
<td>Methaemoglobinaemia</td>
<td>Smoking and increased carboxyhaemoglobin</td>
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<td>Haemoglobins with high affinity for oxygen</td>
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Appropriate polycythaemias (1) are defined by physiologically intact upregulation of Epo production to meet tissue oxygen demand, which then drives augmented erythropoiesis. These disorders may be inherited or acquired conditions that lead to tissue hypoxia. Inappropriate polycythaemias (1) are caused by inappropriate increases of erythropoietin signalling such as seen in congenital or acquired primary polycythaemias, in polycythaemia of disordered hypoxia sensing, in ectopic production of erythropoietin by tumours. Dysregulation of angiotensin II signalling in renal transplant patients might also be considered an inappropriate polycythaemia (2, 3). Polycythaemias can also be classified as being primary or secondary (Table 1B). Primary polycythaemias are characterised by a defect within the progenitors that leads to Epo hypersensitivity or independence, which drives the polycythaemia phenotype. These disorders are characterised by a low Epo level and can be divided into two categories:
1. Congenital gain-of-function mutations of the \( \text{EPOR} \) gene lead to primary familial and congenital polycythaemia (PFCP) wherein the Epo signal is augmented by the mutation.  
2. In PV, a somatic (acquired) mutation located downstream of the physiological stage of EpoR activation, i.e. the \( \text{JAK2V617F} \) gain-of-function mutation, contributes to the pathogenesis.
Secondary polycythaemias are largely Epo-driven disorders. There are however other stimulators of erythropoiesis including cobalt, angiotensin II, and insulin-like growth factor 1 (IGF-1) (2). Some congenital defects lead to dysregulation of Epo production and polycythaemia due to inappropriately high Epo levels. Some of these congenital disorders have features of both primary and secondary polycythaemia (i.e. Chuvash polycythaemia).

2.3 Regulation of erythropoietin production
Epo is the principal regulator of erythropoietin signalling as evidenced by its modulation of erythroid proliferation, prevention of apoptosis, and promotion of erythroid differentiation (reviewed by Krantz (4)). Regulation of Epo production can be mediated by hypoxia or be independent of hypoxia.

2.3.1 Molecular basis of hypoxia sensing
Hypoxia inducible factor transcription factors
Hypoxia influences erythropoiesis, energy metabolism, vasculogenesis, iron metabolism, tumour promotion and other processes (reviewed by Hirota (5) and (6)). The transcription factors, hypoxia inducible factor (HIF)-1 and HIF-2, are induced in hypoxic cells and bind to a cis-acting nucleotide sequence referred to as the hypoxia-responsive element (HRE), first identified in the 3’-flanking region of the human \( \text{EPO} \) gene (7). Many hypoxia-inducible genes are directly regulated by HIFs. Dr. Semenza's
group showed that ~ 3% of all genes expressed in endothelial tissue are HIF-1 regulated (8). HIFs are heterodimeric transcription factors composed of an HIF-α subunit and an HIF-β subunit (Figure 1). The HIF-β subunit belongs to the basic helix-loop-helix (bHLH)-containing PER-ARNT-SIM (PAS)-domain family of transcription factors. Only the HIF-1α and HIF-2α subunits are regulated by hypoxia and exist exclusively in HIF. Thus HIF-α is the key subunit determining the hypoxia-modulated amount and activity of HIF and the transcription of hypoxia-inducible genes.

**HIF-1α**

The half-life of HIF-1α in the cell is only minutes under normoxic conditions, as the protein is rapidly degraded by the VHL protein ubiquitin-proteasome pathway (9).

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**Figure 1: Hypoxic and normoxic control of HIF-1α**

Overview of HIF-1α regulation. The left panel depicts the oxygen dependent pathway. In the middle of the cartoon, in the presence of oxygen, HIF-1α is proline-hydroxylated, which allows interaction with elongins C and E2, and VHL protein leading to ubiquitinisation and destruction of HIF-1α in the proteasome. The left portion of the cartoon depicts the lack of HIF-1α degradation in hypoxia, with formation of HIF-1α and HIF-1β dimer, resulting in transcriptional regulation of HIF controlled genes. The right panel depicts the oxygen independent pathway wherein RACK1 interacting with elongins C and E2 promote ubiquitinisation and destruction of HIF-1α in the proteasome.
This is initiated by posttranslational hydroxylation of proline at position 564 of the HIF-1α molecule by one of several iron-containing proline hydroxylases. Proline 564 hydroxylation facilitates the binding of HIF-1α to VHL protein and subsequent degradation. Under hypoxic conditions, proline 564 of HIF-1α is not hydroxylated. The unmodified protein escapes VHL-binding, ubiquitination, and degradation, but rather is translocated to the cell nucleus where it dimerises with HIF-1β to form HIF-1 and then activates transcription through binding to specific HREs. Another regulatory step involves O2-dependent hydroxylation of asparagine at position 803 of HIF-1α, which requires the enzyme FIH-1 (factor inhibiting HIF-1). Hydroxylation of asparagine 803 during normoxia blocks the binding of two other transcription factors, p300 and CBP, to HIF-1 resulting in inhibition of HIF-1 mediated gene transcription. Under hypoxic conditions, asparagine 803 of HIF-1α is not hydroxylated, p300 and CBP can bind to the HIF-1 dimer, and transcriptional activation of HIF-1 target genes occurs. In summary, the targeting and subsequent polyubiquitination of HIF1α requires pVHL, iron, O2 and proline hydroxylase activity, and, as depicted in Figure 1, this complex constitutes the oxygen sensor (10, 11).

**HIF-2α**

HIF-1α and HIF-2α exhibit high sequence homology but have different mRNA expression patterns. HIF-1α is expressed ubiquitously whereas HIF-2α expression is restricted to certain tissues (5, 12). Both HIF-1α and HIF-2α are regulated through identical mechanism by hypoxia and form a dimer with the same HIF-1β subunit. The kidneys are the main site of Epo production, i.e. renal interstitial cells, and HIF-1 is the principal regulator of EPO transcription in this organ (5). In other tissues, such as the liver (14), which generates ~ 20% of circulating Epo, and the brain (13), EPO gene transcription is HIF-2-regulated (12). The discovery of an iron-responsive element in the 5’ untranslated region of HIF2α reveals a novel regulatory link between iron availability and HIF-2α expression (15) that may impact control of erythropoiesis. Thus, when iron supply is limited, HIF-2α would decrease, and when iron is abundant, hepatic HIF-2α would increase, enhancing liver-synthesised Epo production and promoting erythropoiesis (16).

**Hypoxia independent regulation of HIF**

O2-dependent regulation of the HIF-1α subunit is mediated by prolyl hydroxylases, VHL protein, and a proteosomal complex as described above. A regulatory pathway of HIF that is independent of hypoxia has also been described. This mechanism involves the receptor of activated protein kinase C (RACK1) as an HIF-1α-interacting protein that promotes prolyl hydroxylase/VHL-independent proteosomal degradation of HIF-
1α. RACK1 competes with heat shock protein 90 (HSP90) for binding to the PAS-A domain of HIF-1α. HIF-1α degradation is abolished by RACK1 loss-of-function. RACK1 binds to the proteasomal subunit, elongin-C, and promotes ubiquitination of HIF-1α (Figure 1). Thus, RACK1 is an essential component of an O2/PHD/VHL-independent mechanism for regulating HIF-1α (17).

3. Primary familial polycythaemia (low Epo levels and normal tissue oxygenation)

Primary familial and congenital polycythaemia (PFCP) is the only recognised type of primary familial polycythaemia. It is characterised by an autosomal dominant mode of inheritance, and less frequently, by the occurrence of sporadic cases (18-21). This can be contrasted with Chuvash polycythaemia where the inheritance is autosomal recessive. The clinical features of PFCP include absence of predisposition to the development of acute leukaemia or other myeloproliferative disorders, absence of splenomegaly, normal white blood cell and platelet counts, low plasma Epo levels, normal haemoglobin-oxygen dissociation curve (indicated by a normal P50) and hypersensitivity of erythroid progenitors to exogenous erythropoietin in vitro (20-25). PFCP is generally thought to be a benign condition, but it has been reported to be associated with a predisposition to cardiovascular problems, such as hypertension, coronary artery disease, and cerebrovascular events, and these events are not clearly related to an elevated haematocrit (26-28). Association with cardiovascular disease has not been described in all series.

The distal cytoplasmic region of the EPOR, in association with SHP-1, is required for down-regulation of Epo-mediated activation of JAK2/STAT5 proteins (29, 30). Thus far, more than 10 mutations of the Epo receptor gene (EPOR) have been convincingly linked with PFCP (20-25), 3155. In general, these mutations result in truncation of the EPOR cytoplasmic carboxyl terminal leading to loss of its negative regulatory domain, resulting in a gain-of function of EPOR. Additional missense EPOR mutations have been described that are not clearly linked to PFCP or other disease phenotypes (21, 55). Absence of polycythaemic phenotype in some patients with EPOR mutation is suggestive of a role played by gene modifiers or epigenetic factors in phenotypic penetrance (32).

4. Secondary familial polycythaemia (elevated Epo levels due to tissue hypoxia)

4.1 High oxygen affinity haemoglobin mutants

Affinity of haemoglobin for oxygen is expressed as the P50, which is the partial pressure of oxygen in blood at which 50% of the haemoglobin is saturated with
oxygen. An abnormally low P50 reflects an increased affinity of haemoglobin for oxygen. During oxygenation and deoxygenation, there is considerable movement along the α1/β2 interface (33). Several haemoglobin mutants have substitutions affecting this interface. Other haemoglobin variants have amino acid substitutions involving the C-terminal residues of the β chain or of the 2, 3 BPG binding sites. All these substitutions can affect the cooperative nature of oxygen binding with haem, the change from T to R state and vice versa, and in turn can change the affinity of haemoglobin for oxygen. The majority of mutations affecting oxygen affinity result in high affinity haemoglobin variants that lead to relative tissue hypoxia and compensatory secondary appropriate polycythaemia. There are 91 haemoglobin variants, listed on the globin server, known to be associated with high affinity for oxygen (http://globin.bx.psu.edu/hbvar/menu.html; last accessed December 24, 2008). Thus an autosomal dominant polycythaemia with normal to elevated Epo levels is suggestive of a mutant haemoglobin with high affinity for oxygen (33). The therapy of this compensatory polycythaemia by phlebotomies is ill advised.

Haemoglobin F binds oxygen normally but in the cell has high oxygen affinity due to reduced binding of 2,3-BPG (see below).

4.2 2,3-BPG deficiency
Congenitally low erythrocyte 2,3 biphosphoglycerate (2, 3 BPG) level can occur because of deficiency of the red cell enzyme 2, 3-BPG mutase (34, 35). This is an extremely rare autosomal recessive condition. This disorder should be suspected in the case of isolated polycythaemia (without any feature of myeloproliferative disorders such as progressive increase of RBC mass, high platelet and granulocyte counts and splenomegaly), absence of a family history and low P50 (signifying high oxygen affinity). A haemoglobin mutation needs to be ruled out first. In these cases, the red cells will have high oxygen affinity; however, unlike high affinity haemoglobin, the oxygen affinity of the haemolysate is normal and the level of 2, 3-BPG is very low.

4.3 Congenital methaemoglobinaemias
There are three types of hereditary methaemoglobinaemias (reviewed by Gregg & Prchal (36)). Two are inherited as autosomal recessive traits: cytochrome b5R deficiency and cytochrome b5 deficiency. The third type is an autosomal dominant disorder, haemoglobin M (Hb M) disease in which there is a mutation of one of the globin genes. All congenital methaemoglobinaemias are associated with suboptimal delivery of oxygen per red cell and this may result in compensatory secondary appropriate polycythaemia. The resulting polycythaemia is typically mild and its treatment is not advisable as it would only decrease tissue oxygen delivery and lead to tissue hypoxia.
5. Disorders of hypoxia sensing (elevated Epo levels and normal tissue oxygenation)

5.1 Chuvash polycythaemia

This disorder was the first hereditary condition of augmented hypoxia sensing to be recognised. Chuvash polycythaemia, the only known endemic polycythaemia, is an autosomal recessive hereditary polycythaemia. The Chuvash people reside in the mid-Volga River region in Russia where Chuvash polycythaemia affects hundreds of people, making it the most common congenital polycythaemia (37). Outside of Chuvashia, this form of familial polycythaemia has also been found sporadically in diverse ethnic and racial groups (38-40), and a high prevalence of this disorder was reported in the Italian island of Ischia (41). In a study of five Chuvash families with polycythaemia, a mutation of \( VHL \ (598C \rightarrow T) \) was found in the affected individuals (42). This mutation impairs the interaction of pVHL with HIF-1\( \alpha \), thus reducing the rate of ubiquitin-mediated destruction of HIF-1\( \alpha \). As a result, the level of the HIF-1 increases and leads to increased expression of target genes including endothelin-1, \( EPO \), plasminogen activator inhibitor (PAI), transferrin, transferrin receptor and \( VEGF \) (42-45). The \( VHL598C \rightarrow T \) mutation also impairs the interaction of pVHL with HIF-2\( \alpha \) (46), and an animal model of the Chuvash polycythaemia mutation indicates that it may be increased levels of HIF-2\( \alpha \) rather than HIF-1\( \alpha \) that mediate the increased Epo levels and polycythaemia in this condition (47). Chuvash polycythaemia is characterised by an intact response to hypoxia despite increased basal expression of a broad range of hypoxia-regulated genes in normoxia. Unexpectedly, the erythroid progenitors are hypersensitive to Epo; thus Chuvash polycythaemia shares features of primary as well as secondary polycythaemia.

Chuvash polycythaemia is a unique VHL syndrome characterised by homozygous germline mutation of \( VHL \) leading to predisposition to development of varicose veins, benign vertebral haemangiomas, low systemic blood pressures, pulmonary hypertension, thrombosis, bleeding, cerebral vascular events, and increased mortality (Table 2). Peripheral blood profiling indicates lower white blood cell and platelet counts than controls, lower CD4 counts, elevated levels of both pro-inflammatory and anti-inflammatory cytokines, and altered plasma thiol levels with elevated homocysteine and glutathione and low cysteine concentrations (44, 45, 48-50). Despite increased expression of HIF-1\( \alpha \) and VEGF in normoxia, Chuvash polycythaemia patients do not display a predisposition to tumour formation. Imaging studies of 33 Chuvash polycythaemia patients revealed unsuspected cerebral ischaemic lesions in 45% but no tumours characteristic of VHL syndrome (44). It is not known whether therapy with aspirin or phlebotomy in patients with Chuvash polycythaemia prevents complications.
### Table 2: Characteristics of Chuvash polycythaemia

**Molecular**
- Homozygous $VHL598C\rightarrow T$
- Decreased capture of HIF-α by VHL protein
- Decreased degradation of HIF-α by the ubiquitin-proteasome pathway
- Increased HIF-α under normoxic conditions
- Altered transcription of multiple HIF-regulated genes

**Increased plasma concentrations of products of HIF-regulated genes**
- Endothelin-1
- Erythropoietin
- Plasminogen activator inhibitor-1
- Vascular endothelial growth factor
- Transferrin
- Transferrin receptor

**Physical findings**
- Ruddy complexion
- Varicose veins, increased prevalence
- Heart murmur, increased prevalence
- Clubbing of digits, increased prevalence
- Lower systemic blood pressure
- Higher pulmonary artery pressure on echocardiogram
- Benign vertebral haemangiomas on MRI
- Cerebral ischaemic lesions on MRI

**Complete blood count**
- Polycythaemia (erythrocytosis)
- Lower white blood cell count
- Lower platelet count

**Immunologic profile**
- Increased plasma concentrations of Th-1 cytokines (GM-CSF, IFN-γ, IL-2 and IL-12, TNF-α)
- Increased plasma concentrations of Th-2 cytokines (IL-4, IL-5, IL-10, IL-13)
- Normal ratio of Th-1 to Th-2 cytokines
- Lower CD4 counts

**Thiol metabolism**
- Increased plasma concentrations of homocysteine, glutathione and cysteinylglycine
- Reduced plasma concentration of cysteine

**Clinical course**
- No increase in incidence of renal cell carcinoma, pheochromocytoma or haemangioblastoma
- Increased peptic ulcers
- Increased major thromboses
- Increased haemorrhage
- Increased cerebrovascular accidents
- Increased mortality
Homozygosity for \(VHL\ 598C \rightarrow T\) occurs sporadically in Caucasians in the United States and Europe and in people of Southeast Asian (Indian subcontinent) ancestry (38-41). Thrombotic complications have been reported in some of these individuals.

To address the question of whether the \(VHL\ 598C \rightarrow T\) substitution occurred in a single founder or resulted from recurrent mutational events, haplotype analysis was performed on subjects bearing the \(VHL\ 598C \rightarrow T\) mutation and normal unrelated individuals from Chuvash, Asian, Caucasian, Hispanic and African-American ethnic groups (51). These studies indicated that in most individuals, the \(VHL\ 598C \rightarrow T\) mutation arose in a single ancestor between 12,000 and 51,000 years ago. However, a polycythaemic family in Turkey had a different haplotype indicating that the \(VHL\ 598C \rightarrow T\) mutation in this family occurred independently (52).

In contrast, autosomal dominant mutations of the \(VHL\) gene cause VHL syndrome (53). Heterozygotes for dominant \(VHL\) mutations are at increased risk of developing haemangioblastomas, renal cell carcinoma, pheochromocytoma, pancreatic endocrine tumours, and endolymphatic sac tumours when they acquire a somatic mutation in the normal \(VHL\) allele. Some patients with VHL syndrome also develop acquired polycythaemia (53). Increased expression of HIF-1 and possibly VEGF may underlie the VHL tumour predisposition syndrome and the development of haemangioblastoma and renal cell carcinoma. However, the absence of a predisposition to tumourigenesis in Chuvash polycythaemia patients implies that deregulation of HIF-1 and VEGF may not be sufficient to cause predisposition towards tumour formation in VHL syndrome.

Most heterozygotes for \(VHL598C \rightarrow T\) do not have polycythaemia. However, an English patient has been described who was a heterozygote for \(VHL\ 598C \rightarrow T\) (40), although the inheritance of a deletion of a \(VHL\) allele or a null \(VHL\) allele in a trans position was not excluded in this patient. Subsequently, two \(VHL\) heterozygous patients with polycythaemia were described in whom a null \(VHL\) allele was more rigorously excluded (52, 54); one of these patients also had ataxia-telangiectasia (54). Some patients with congenital polycythaemia have proven to be compound heterozygotes for the Chuvash mutation, \(VHL\ 598C \rightarrow T\), and other \(VHL\) mutations including \(562C \rightarrow G, 574C \rightarrow T, 388C \rightarrow G,\) and \(311G \rightarrow T\) (39, 52, 54).

### 5.2 Non-Chuvash germline VHL mutations

Non-Chuvash germline \(VHL\) mutations also cause polycythaemia. A Croatian boy was homozygous for \(VHL\ 571C \rightarrow G\), the first example of a homozygous \(VHL\) germline mutation other than \(VHL\ 598C \rightarrow T\) causing polycythaemia (39). Additionally, a Portuguese girl was a compound heterozygote for \(VHL\ 562C \rightarrow G\) and \(VHL\ 253C \rightarrow T\) (54, 55). A few cases of congenital polycythaemia with mutations of only one non-Chuvash polycythaemia \(VHL\) allele have been described. Two Ukrainian children with
polycythaemia were heterozygotes for VHL \(376G\rightarrow T\), but the father with the same mutation was not polycythaemic (38). Peripheral blood erythroid progenitors from the children and father were hyper-responsive to recombinant Epo in \textit{in vitro} clonogenic assays in a way similar to that seen in Chuvash polycythaemia patients.

### 5.3 Proline hydroxylase deficiency

A family with a proline hydroxylase domain protein 2 (PHD2) mutation (950C→G) was recently described in which heterozygotes for this mutation have mild or borderline polycythaemia (56). Since then, four additional patients with unexplained polycythaemia who are heterozygote carriers of different mutations in PHD2 have been reported (two frameshift mutations, 606delG and 840_841insA, both located in exon 1, and two nonsense mutations, 1112G→A and 1129C→T, in exon 3) (57, 58). One of these patients had a major thrombotic event (thrombosis of the sagittal sinus) (58). These observations underscore the importance of defective HIF signalling in the genesis of polycythaemic disorders.

### 5.4 HIF-2\(\alpha\) gain-of-function mutations

Recently, a family was described in which members with polycythaemia were heterozygous carriers of a HIF-2\(\alpha\) Gly537Trp mutation which served to stabilise the HIF-2\(\alpha\) protein (59). The same group subsequently reported four additional polycythaemic patients with heterozygous Met535Val or Gly537Arg mutations in the HIF-2\(\alpha\) gene (60). The patients tend to present at a young age with elevated serum Epo. These findings support the importance of the proline hydroxylase, HIF-2\(\alpha\), VHL axis in human Epo regulation and in the pathogenesis of familial anaemias due to abnormal hypoxia sensing.

### 5.5 Congenital polycythaemias of yet unidentified defect with elevated or inappropriately normal levels of Epo

Not all patients with congenital polycythaemias with normal or elevated Epo levels do have \textit{VHL}, PHD2 or HIF-2\(\alpha\) mutations, and the molecular basis of polycythaemia in these cases remains to be elucidated. It is not clear why in some families, the polycythaemia is dominantly inherited (61), in others recessively, and in some it is sporadic and, why in families with the same mutation the phenotype can be different (55, 62). Lesions in genes linked to hypoxia-independent regulation of HIF as well as oxygen-dependent gene regulation pathways are leading candidates for mutation screening in polycythaemic patients with normal or elevated Epo without \textit{VHL}, PHD2 or HIF-2\(\alpha\) mutations.

### 6. Familial polycythaemia vera and other myeloproliferative disorders

There is growing evidence that the acquired primary polycythaemic disorder
polycythaemia vera, which is caused by somatic mutation(s), often has a familial predisposition (63); in some families other myeloproliferative disorders are also present (64-65). It is estimated that about 5% of PV patients have relatives with PV or with other myeloproliferative disorders (65). This suggests the existence of germ-line mutations that either facilitate the somatic mutation(s) or contribute to somatic acquired mutations to result in familiar clustering of myeloproliferative disorders. The molecular basis of these interactions yet remains to be defined. This suggests that these families inherit a yet-to-be identified gene(s) that increases the acquisition of PV somatic mutation(s).

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Multiple Choice Questionnaire

To find the correct answer, go to http://www.esh.org/iron-handbook2009answers.htm

1. Mutation of which of the following genes has not been associated with congenital polycythaemia?
   a) VHL .................................................................
   b) HIF-2 ..............................................................
   c) EPOR ..............................................................
   d) EPO ..............................................................

2. Chuvash polycythaemia is caused by homozygosity for the 598C→T mutation in the VHL gene. Which of the following is a recognised manifestation of Chuvash polycythaemia?
   a) Benign vertebral hemangiomas ................................
   b) Pheochromocytoma ...........................................
   c) Hypertension ....................................................
   d) CNS cerebellar haemangioblastoma ....................

3. Which of these polycythaemias is caused by a somatic mutation(s)?
   a) Primary familial congenital polycythaemia ..........
   b) HIF-2 mutation ................................................
   c) Polycythaemia vera ..........................................  
   d) Chuvash polycythaemia .................................

4. Which of these congenital polycythaemic states is associated with low erythropoietin level and augmented erythropoietin signaling?
   a) Primary familial congenital polycythaemia (due to EPO receptor mutations) ................................
   b) Chuvash polycythaemia (598C→T VHL mutation)  
   c) HIF-2 mutation .................................................
   d) Proline hydroxylase mutation ............................

5. Which of these polycythaemia states is inherited as an autosomal dominant disorder?
a) Polycythaemia vera
b) Chuvash polycythaemia
c) Mutations of globin gene causing high haemoglobin oxygen affinity
d) Cytochrome b5 reductase mutations causing methaemoglobinaemia and polycythaemia