Somatic mutations of *JAK2* exon 12 as a molecular basis of erythrocytosis

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The human erythron is a highly specialized tissue that is responsible for oxygen transport.[1] It includes the erythroid marrow, where red cells are produced, circulating red cells, the reticuloendothelial system, where red cells are phagocytosed at the end of their life span, and the erythropoietin-producing cells in the kidney. Within the erythroid marrow, differentiation and maturation of erythroid progenitors and precursors are controlled by several peptides. The most important one is erythropoietin, which is primarily made by a single organ, the kidney, outside the bone marrow and participates in a classic negative feedback control system (Figure 1). In the kidney, erythropoietin production is restricted to specific subsets of cells, i.e., interstitial fibroblast-like cells, and hypoxia is the fundamental physiological stimulus that induces expression of the erythropoietin gene (*EPO*) in these cells.[2]

Hypoxia inducible factor-1 (HIF-1) is the principal factor responsible for transcriptional activation of *EPO*.[3,4] HIF-1 is a heterodimeric transcription factor composed of HIF-1α and HIF-1β subunits, and functions as a master regulator of oxygen homeostasis activating hundreds of genes.[2,4] As illustrated in Figure 2, at normal oxygen tension, HIF-1α rapidly undergoes hydroxylation of proline residues by prolyl hydroxylase domain protein 2 (PHD2).[5] The proline hydroxylated HIF-1α interacts with the von Hippel–Lindau protein (VHL), and this binding leads to ubiquitination and proteasomal degradation. At low oxygen tension, by contrast, HIF-1α is stable and forms a heterodimer with HIF-1β, generating the HIF-1 molecule. The heterodimer translocates into the nucleus, binds to response elements in hypoxia inducible genes, and activates their transcription. Thus, hypoxia rapidly increases renal production of erythropoietin through activation of HIF-1-mediated transcription of the *EPO* gene in interstitial erythropoietin-producing cells.

Erythropoietin exerts its effects by binding to a surface receptor (EPO-R) present on erythroid progenitors and precursors.[6] While only a few receptors are found on early burst-forming units-erythroid (BFU-E), their number increases with differentiation and a peak of more than 1,000 receptors/cell is reached at the colony-forming unit-erythroid (CFU-E) and proerythroblast stages.[7] EPO-R transduces the erythropoietin signal by activating members of the Janus kinase (JAK) family of proteins, which phosphorylate cytoplasmic targets, including the signal transducers and activators of transcription (STAT). JAK2 plays an essential role in hematopoiesis by mediating signals from several hematopoietic growth factors, including erythropoietin, thrombopoietin and granulocyte colony-stimulating factor (G-CSF). Erythropoietin mainly acts as a survival factor for erythroid cells with high receptor number, i.e. CFU-E and proerythroblasts.

The term *erythrocytosis* defines any condition characterized by increased values of hemoglobin, hematocrit and red blood cells. Absolute erythrocytosis is characterized by the presence of an increased red cell mass, while relative erythrocytosis is secondary to plasma volume depletion. According to its Greek etymology, *polycythemia* means *many cells*, and therefore this term should be used to define conditions characterized not only by erythrocytosis, but also by leukocytosis and/or thrombocytosis.

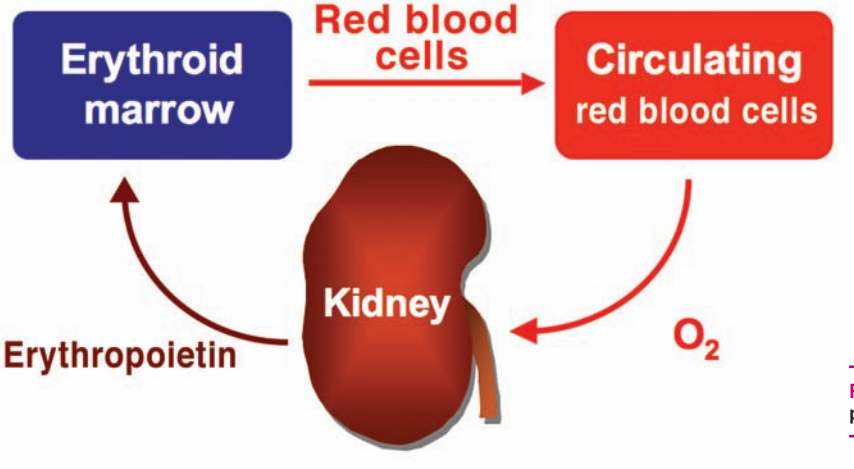

Figure 1. Feedback regulation of red cell production.

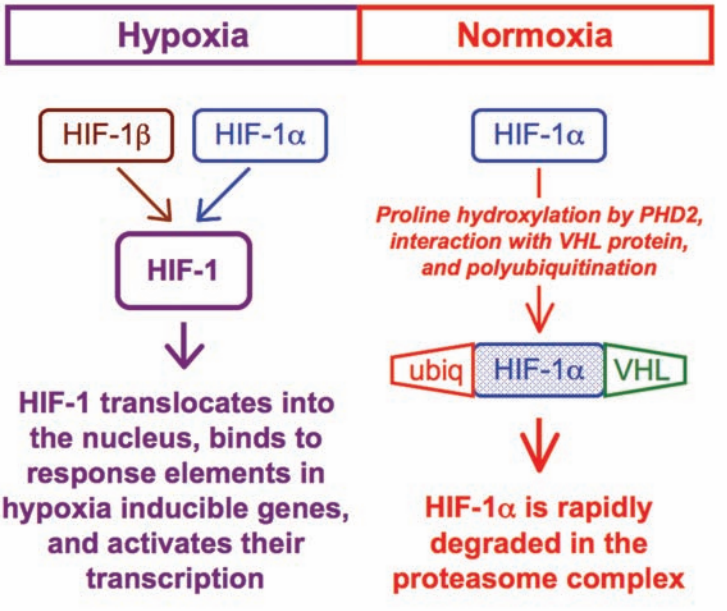

Figure 2. Schematic representation of the HIF-1 oxygen-sensing pathway.

The measurement routinely employed for identifying erythrocytosis is hemoglobin concentration, whose upper normal limit varies according to several factors, including age, sex, ethnic group, and altitude at which the subject lives. Arbitrary upper limits may be 17 g/dL for the adult male and 16 g/dL for the adult female at sea level.[1] The fact that a hemoglobin level is above these limits does not necessarily mean that it is abnormal and the subject has erythrocytosis. Since there are overlaps between normal individuals and patients with respect to hemoglobin level,[1] the interpretation of an individual value that lies outside the reference range should rely upon a probability calculation. In general, the higher the hemoglobin level, the more likely it is that the value represents erythrocytosis. More stringent limits would, therefore, be those adopted by the WHO for the diagnosis of polycythemia vera, i.e., 18.5 g/dL in men, 16.5 g/dL in women, or the 99th percentile of a method-specific ref-

Table 1. Classification of erythrocytosis due to germline or somatic mutations of genes involved in the regulation of erythropoiesis.

Condition	*Molecular mechanisms*	*Features*
Hereditary conditions		
Familial erythrocytosis type 1 (OMIM 133100)	Germline mutations in the *EPOR* gene: mutated receptors transduce erythropoietin signal more efficiently	Autosomal dominant, low serum erythropoietin
Familial erythrocytosis type 2, or Chuvash or VHL-dependent polycythemia (OMIM 263400)	Germline mutations in the *VHL* gene (mainly R200W missense mutation): the mutated VHL protein is less efficient in binding HIF-1α, and therefore in determining its degradation	Autosomal recessive, normal to increased serum erythropoietin
Familial erythrocytosis type 3 (OMIM 609820)	Germline mutations in the *EGLN1* gene encoding PHD2: the mutated PHD2 protein is less efficient in hydroxylating proline residues of HIF-1α, and therefore in determining its degradation	Autosomal dominant, normal serum erythropoietin
Erythrocytosis associated with high-oxygen affinity hemoglobin	Germline mutations in α or β globin genes	
Acquired conditions		
Polycythemia vera	Gain-of-function, somatic mutation V617F of *JAK2*	Erythrocytosis frequently associated with leukocytosis and/or thrombocytosis; low serum erythropoietin
Polycythemia vera/idiopathic erythrocytosis	Gain-of-function, somatic mutations of *JAK2* exon 12	Isolated erythrocytosis initially, low serum erythropoietin

erence range for age, sex, and altitude of residence.[8]

A classification of absolute erythrocytosis was previously reported in this journal.[9] Table 1 reports a classification of absolute erythrocytosis due to germline or somatic mutations of genes involved in the regulation of erythropoiesis. While germline mutations are responsible for hereditary conditions,[10-12] the somatic ones are typically found in acquired myeloproliferative disorders.

By using sensitive assays, the gain-of-function *JAK2* (V617F) mutation – which involves exon 14 of the gene – can be detected in the vast majority of patients with sporadic polycythemia vera,[13-15] but only in about half of those with familial polycythemia vera.[16] This unique mutation is rarely found in patients with the so-called *idiopathic erythrocytosis*,[17] a poorly defined condition characterized by erythrocytosis of undefined origin. According to Wikipedia, idiopathic is an adjective used primarily in medicine meaning *arising spontaneously or from an obscure or unknown cause*. This term derives from the Greek *idios* – which means one's own – and *pathos* – which means disease in this context. Thus, idiopathic basically means *a disease of its own kind*. In other words, this adjective defines physicians' lack of knowledge.

Somatic mutations of *JAK2* exon 12 have been recently found in patients with either polycythemia vera or idiopathic erythrocytosis who do not carry the unique exon 14 mutation (Table 2).[18-21] Compared with patients with *JAK2* (V617F)-positive polycythemia vera, patients with polycythemia vera carrying exon 12 mutations have a clinical phenotype mainly characterized by isolated erythrocytosis. In this issue of the journal, Percy and co-workers[22] and Martinez-Aviles and co-workers[23] report interesting additional observations on *JAK2* exon 12 mutations in patients with idiopathic erythrocytosis. In the British study,[22] these mutations were detected in a proportion of patients with low serum erythropoietin levels, all of whom had erythropoietin-independent erythroid progenitors. Two of the three Spanish patients with idiopathic erythrocytosis carrying exon 12 mutations also had very low serum erythropoietin

Table 2. *JAK2* exon 12 mutations reported so far.[18-24]

Mutation	*Condition*
F537-K539delinsL	Idiopathic erythrocytosis, polycythemia vera
H538QK539L	Polycythemia vera
H538-K539delinsL	Idiopathic erythrocytosis
K539L	Idiopathic erythrocytosis , polycythemia vera
I540-E543delinsMK	Polycythemia vera
R541-E543delinsK	Idiopathic erythrocytosis, polycythemia vera
N542-E543del	Idiopathic erythrocytosis, polycythemia vera
E543-D544del	Idiopathic erythrocytosis
V536-I546dup11	Polycythemia vera
F537-I546dup10+F547L	Polycythemia vera

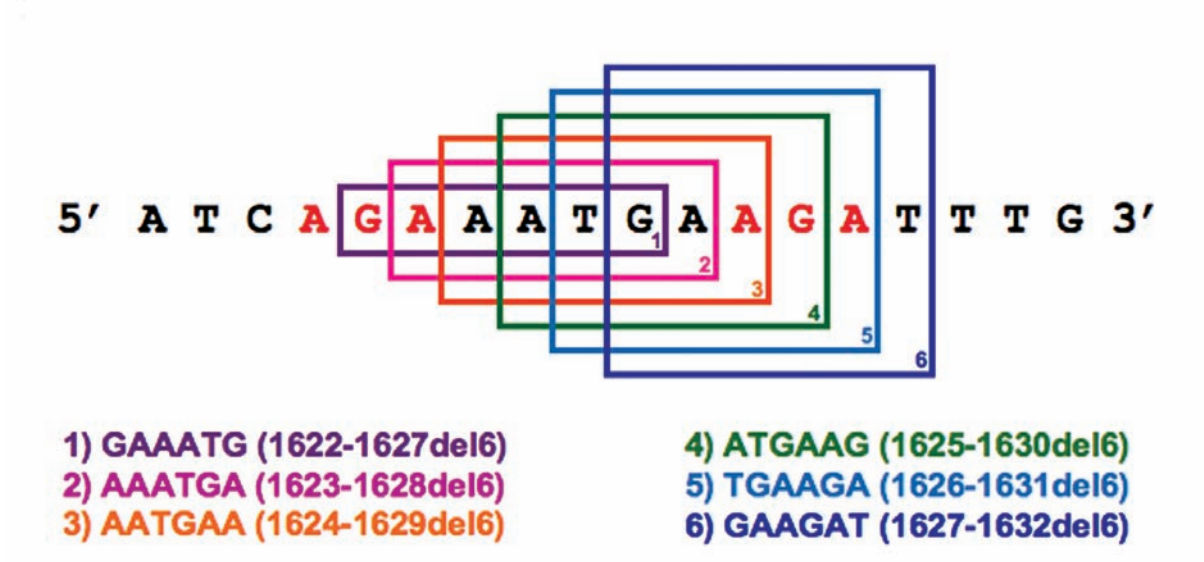

Figure 3. Hypothetical mechanism of slippage mispairing involving the AGA repeats in exon 12 (shown in red) and leading to 6 bp deletions (see text for details concerning the mechanisms that can lead from slippage mispairing to the loss of 6 bp fragments in a hemopoietic stem cell). Numbers from 1 to 6 indicate possible deletion breakpoints, which may lead to R541-E543delinsK (1622-1627del6), N542-E543del (1623-1628del6 or 1624-1629del6), and E543-D544del (1625-1630del6 or 1626-1631del6 or 1627-1632del6). (*Courtesy of Daniela Pietra, Angela Brisci and Laura Cremonesi*)

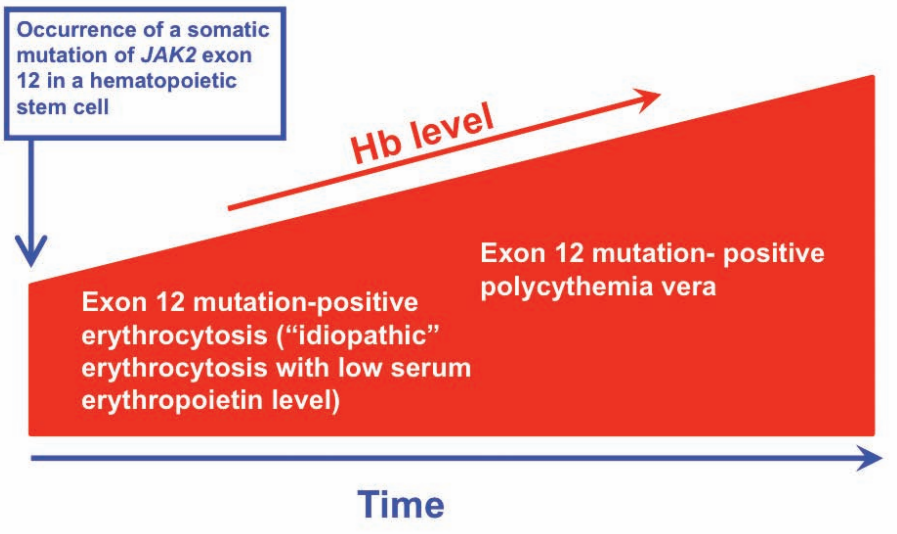

Figure 4. Schematic representation of the clinical course of the human myeloproliferative disorder associated with *JAK2* exon 12 mutations and characterized by isolated erythrocytosis, at least initially. The occurrence of a *JAK2* exon 12 mutation in a hematopoietic stem cell gives rise to a clone. The increased red cell production in turn leads to erythrocytosis. This condition may be relatively stable for years or may progress. According to hemoglobin level and other WHO criteria, this disorder may be diagnosed as idiopathic erythrocytosis or polycythemia vera. Progression to full-blown polycythemia vera – characterized by thrombocytosis and/or leukocytosis – may occur during the clinical course, and extramedullary disease may eventually develop.

levels.[23] Thus, these patients have a myeloproliferative disorder mainly characterized by autonomous overproduction of red cells associated with secondary suppression of erythropoietin synthesis. This pattern has been recently observed by us[24] also in patients with polycythemia vera carrying exon 12 mutations.

There is considerable discrepancy in the frequency of exon 12 mutations in patients with *JAK2* (V617F)-negative polycythemia vera between three studies[18,19,21] in which virtually all patients were found to be positive, and others[20,23] in which less than half of the patients were so. One source of the discrepancy is likely the detection method employed. In any given tissue, a somatic mutation is found in a variable proportion of alleles. Exon 12 mutations are likely associated with small clones initially, and therefore present in small percentages of granulocyte alleles. For their detection, allele-specific PCR is definitely superior to direct sequencing, whose sensitivity is about 10% of mutant alleles. A second crucial issue is the source of DNA for mutation detection. The available evidence suggests that exon 12 mutations may preferentially lead to expansion of erythroid cells within the bone marrow. Therefore, endogenous erythroid colonies appear to be more suitable sources of DNA than granulocytes for detection of these mutations. These considerations clearly indicate that there is a need for highly sensitive, reliable and hopefully relatively simple methods for identifying *JAK2* exon 12 mutations in patients with erythrocytosis.

So far, ten mutations of *JAK2* exon 12 have been reported (Table 2), the most frequent ones being deletions that result in the loss of a 6 bp fragment. They do not disrupt the gene reading frame, but rather alter the final amino acid composition by removing two residues: N542-E543 and E543-D544del are the most frequent of these deletions. Analysis of the sequence across deletion breakpoints shows the presence of two short direct repeats (AGA) in the region involved in these rearrangements (Figure 3). This might facilitate the generation of single-stranded looped intermediates that might be either excluded by replication or cleaved through an enzymatic excision-repair mechanism, resulting in the loss of 6 bp fragments.

The occurrence of the same *JAK2* exon 12 mutations in polycythemia vera and idiopathic erythrocytosis suggests that these conditions are simply different stages of the same *erythrocytosic* myeloproliferative disease (Figure 4). Patients with small mutant clones and marginally elevated hemoglobin levels would be categorized as having idiopathic erythrocytosis, while those with larger clones and hemoglobin levels above the WHO classification limits[8] would meet the WHO diagnostic criteria for polycythemia vera. A few observations suggest that some of these latter patients may also have thrombocytosis and/or leukocytosis. Whether this reflects clone size, genetic background, or co-operating mutations remains to be established.

There is no doubt that the identification of *JAK2* (V617F) has opened avenues of research, along which neglected Cinderellas – Philadelphia-negative myeloproliferative disorders – have achieved triumphant rewards. It is to be hoped that the adjective idiopathic will become obsolete in the forthcoming molecular hematology era. Some patients currently diagnosed with a idiopathic erythrocytosis will be diagnosed with hereditary disease or myeloproliferative disorder. Others will be simply defined as normal individuals with constitutively high hemoglobin levels, perhaps related to gene polymorphisms.

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