

Review

# Familial erythrocytosis arising from a gain-of-function mutation in the *HIF2A* gene of the oxygen sensing pathway

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## RED CELL HOMEOSTASIS

Red blood cell production and the supply of oxygen to the tissues is tightly controlled by a negative feedback mechanism involving the kidney. Any fall in the level of oxygen is sensed by the kidney, which then synthesizes the erythropoietin (Epo). This growth factor acts upon bone marrow erythroid precursors to increase the number of circulating red blood cells thus correcting the oxygen deficit. The *EPO* gene, which encodes erythropoietin, is transcriptionally regulated by the Hypoxia Inducible Factor (HIF) transcription complex. The HIF complex is composed of two different subunits, alpha and beta (also known as ARNT), both constitutively expressed. The alpha subunit is continually synthesized but in the presence of oxygen it is undetectable due to degradation by the proteasome. A family of enzymes, prolyl hydroxylase domain proteins (PHD) of which there are 3 members (PHD1, PHD2 and PHD3), are able to hydroxylate key prolines in the oxygen dependent degradation (ODD) domain of HIFalpha and these enzymes are only active in the presence of oxygen (Fig 1). The von Hippel Lindau (VHL) protein is able to associate with HIFalpha once it is hydroxylated and then molecules of ubiquitin are added. This is the signal that sends HIFalpha to the proteasome for degradation. As the level of oxygen falls the PHD enzymes are no longer active resulting in less and less HIFalpha being degraded (Fig 1). Consequently, all the genes under the control of the HIF transcription complex, which includes *EPO*, are elevated. Once the oxygen deficit is

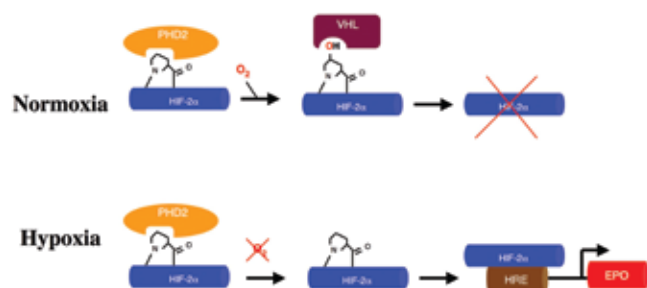


Fig 1. The control of erythropoietin synthesis by the oxygen sensing pathway.

In the presence of oxygen HIFalpha is hydroxylated by the prolyl hydroxylase PHD2 and once modified the von Hippel Lindau (VHL) protein is able to bind. HIFalpha is then targeted to the proteasome. In the absence of oxygen HIFalpha is not hydroxylated and VHL does not associate thereby allowing HIFalpha to bind to hypoxia response elements (HRE) in target genes such as *EPO*.

corrected by the enhanced production of red blood cells the oxygen tension rises and HIFalpha is increasingly degraded.

## EYTHROCYTOSIS

Erythrocytosis is a rare red cell disorder that can arise from diverse molecular origins. It is characterised by an elevated haematocrit and haemoglobin level. There is no accompanying increase in the number of white cells or platelets. Both sporadic and familial forms exist and age of presentation is highly variable. Individuals with erythrocytosis exhibit a wide range of serum Epo levels and this reflects the heterogeneity of this disorder.

Over the last decade a registry of erythrocytosis individuals has been established at the Belfast City Hospital to which patients from the UK and Ireland been referred<sup>1</sup>. Interestingly, there is a preponderance of males with a ratio of 1.7 males to every female<sup>2</sup>. The mean age of erythrocytosis individuals present on the data base is 37 years<sup>2</sup>. Most individuals have inappropriately normal (46%) or raised (26%) serum Epo levels<sup>2</sup> indicating that a significant cause of erythrocytosis is dysregulation of Epo synthesis via the oxygen sensing pathway.

Investigation of the VHL and PHD2 proteins in erythrocytosis individuals has detected several different mutations. One VHL mutation, Arg200Trp predominates, and was first detected in a population in the Chuvash region of Russia<sup>3</sup>. Further studies revealed its worldwide distribution and the possibility of a common origin for most cases of this mutation<sup>4</sup>. In contrast, only a few different PHD2 mutations have been<sup>5-7</sup>. However, there remains a large cohort of erythrocytosis individuals who also possibly have an underlying defect in the oxygen sensing pathway. Previous investigation of HIF-1alpha had not revealed any causative mutations<sup>8</sup> but we set about investigating HIF-2alpha as murine studies indicated this isoform was the major controller of Epo synthesis<sup>9</sup>.

## THE GLY537TRP HIF-2ALPHA MUTATION

Screening the ODD region of HIF-2alpha detected a heterozygous base change of G to T at base 1609 in exon 12 (Fig 2) in a young man as reported in the New England Journal

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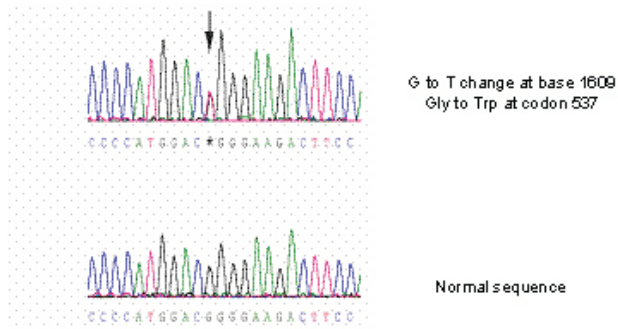


Fig 2. Detection of the c.1609G>T mutation in exon 12 of HIF2A.

Exon 12 was amplified by polymerase chain reaction (PCR) from DNA extracted from peripheral blood. The PCR product was sequenced and replacement of G with T at 1609 was discovered as indicated by arrow.

Bases are as follows: G = black; A = green; T = red; C = blue.

of Medicine<sup>10</sup>. This mutation resulted in the replacement of the amino acid glycine at position 537 with tryptophan. Screening a group of health control samples did not detect this same mutation suggesting it may be associated with erythrocytosis. The proband presented with erythrocytosis at the age of 23 years exhibiting an elevated haemoglobin level of 21.7 g/dL and a haematocrit of 0.64. Further investigation of his family revealed that both his mother and maternal grandmother also had erythrocytosis presenting at the ages of 35 and 54 years respectively (Fig 3). Mutation analyses confirmed both these individuals also possessed the Gly537Trp mutation. Measurement of serum Epo levels in the three erythrocytosis individuals revealed they were well above the normal range for the assay. The platelet and white cell counts for all three erythrocytosis family members were normal. Other non-affected family members did not possess the Gly537Trp mutation.

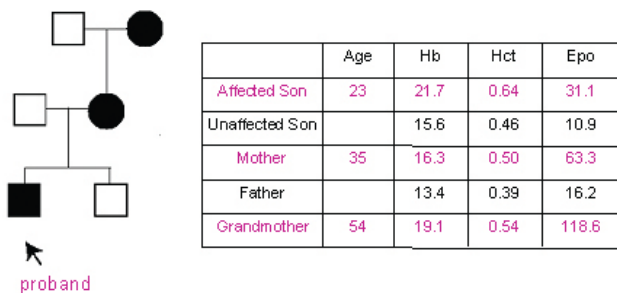


Fig 3. Pedigree of family with hereditary erythrocytosis.

Three generations of one family were found to have erythrocytosis and the first noted case is indicated by an arrow. Squares and circles represent male and female family members respectively, while solid symbols indicate erythrocytosis individuals. Included in a table are salient haematological features at the time of presentation and a recent serum erythropoietin level.

### GLY537TRP HIF-2ALPHA FUNCTIONAL STUDIES

To confirm whether the Gly537Trp mutation could indeed result in erythrocytosis recombinant protein was prepared from a bacterial plasmid containing a DNA copy of the mutant gene. Using this recombinant protein assays were performed to discover if the mutation affected the function of HIF-2alpha. Less association of PHD2 with HIF-2alpha

was detected which would affect the ability of PHD2 to hydroxylate HIF-2alpha. The hydroxylation of HIF-2alpha is important for the association of VHL so we wanted to establish if the mutant protein was hydroxylated to the same level as the wild type protein. We found that the mutant was less hydroxylated and subsequently detected less association with its binding partner VHL. We were also able to show that the Gly537Trp HIF-2alpha protein was more stable than wild type and was able to up-regulate HIF-2alpha target genes. Thus it could be inferred that Epo synthesis would also be elevated.

The amino acid sequence of HIF-2alpha is highly conserved and Gly537 is present in this isoform from other species such as mouse, chicken and frog but is absent in the other two isoforms of HIFalpha. The replacement of the structurally small glycine with the bulkier tryptophan amino acid would cause disruption of the ODD region. This would prevent optimal binding of PHD2, thus reducing hydroxylation and the association of VHL, resulting in decreased ubiquitinylation of HIF-2alpha (Fig 4). Consequently, HIF target genes would be transcribed at a higher rate.

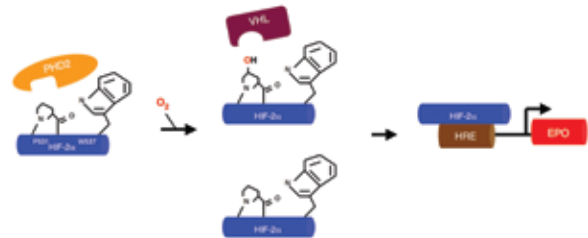


Fig 4. The impact of the Gly537Trp mutation on the function of HIF-2alpha.

The replacement of glycine with a large amino acid would prevent both the association of PHD2 and VHL to HIF-2alpha. Consequently neither hydroxylation or ubiquitinylation of HIF-2alpha would occur. HIF-2alpha is able to evade proteasomal degradation and bind to HRE of EPO to increase transcription.

### SUMMARY

A mutation of HIF-2alpha has been detected in three generations of a family with erythrocytosis and the mutation co-segregated with the erythrocytosis phenotype. Functional studies revealed that Gly537Trp mutation would significantly impair the function of HIF-2alpha thus leading to increased synthesis of Epo. In addition to VHL and PHD2 a further member of the oxygen sensing pathway, namely HIF-2alpha, can be a cause of erythrocytosis. Furthermore, HIF-2alpha plays an important role in the regulation of Epo production. Continued study of idiopathic cases of erythrocytosis with raised serum Epo will reveal whether HIF-2alpha will be a major cause of erythrocytosis on par with VHL.

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The author has no conflict of interest.

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