Red Cell Disorders

Congenital polycythemia with homozygous and heterozygous mutations of von Hippel-Lindau gene: five new Caucasian patients

We report on five Caucasian patients with congenital polycythemia and mutations of the von Hipple-Lindau (*VHL*) gene: a compound heterozygote for the novel exon 1 (*VHL 235C→T*) and previously reported *VHL 562C→G* mutations; three homozygotes for Chuvash *VHL 598C→T* mutation; and a heterozygote for *VHL 523A→G* mutation who also has ataxia-telangiectasia; a rare autosomal disease of childhood onset.

haematologica 2005; 90:128-129
http://www.haematologica.org/journal/2005/1/128

An essential function of red blood cells is to deliver oxygen. Hypoxia sensing is crucial for energy metabolism, angiogenesis, erythropoiesis and various other functions. Hypoxia-inducible factor 1 (HIF1), a heterodimeric transcription factor comprising a HIF-1α subunit and HIF-1β (also known as the aryl hydrocarbon receptor nuclear translocator (ARNT),[1] plays a central role in the response to hypoxia. The HIF-1α subunit is regulated by oxygen. In normoxia its proline is hydroxylated and binds to the von Hippel-Lindau (VHL) protein and is rapidly degraded by ubiquitinization.[1] In hypoxia, HIF-1α is stable and forms a heterodimer with HIF-1β which results in the activation of an array of genes expression of proteins such as those involved in energy metabolism, vasculogenesis (e.g. vascular endothelial growth factor), iron metabolism (e.g. transferrin receptor) and erythropoiesis.[1]

Polycythemia is defined as an increased red cell mass. Primary polycythemia is characterized by an augmented response of erythroid progenitors to erythropoietin (Epo) due to acquired somatic or inherited germline mutations expressed within hematopoietic progenitors which cause erythrocyte overproduction. In secondary polycythemias the erythroid progenitors respond normally to Epo and increased levels of circulating factors driving erythropoiesis, e.g. Epo, IGF1, cobalt. Both primary and secondary polycythemias can be congenital or acquired.[2] Chuvash polycythemia (CP) is endemic in the Chuvash region of Russia. It shares features of both primary and secondary polycythemias, and is due to homozygosity for the 598 C→T mutation in the *VHL* gene.[3] CP has also been found in diverse ethnic and racial groups.[4-6] Rare polycythemic subjects are heterozygous for a *VHL* mutation. In this report we describe five additional polycythemic patients with *VHL* mutations. The 5 new polycythemic patients have 4 different *VHL* mutations. Four of the patients have both *VHL* alleles mutated, one is a heterozygote for a missense VHL mutation (Table 1).

Patient #1 is a compound heterozygote with a novel *235 C→T* (*R79C*) *VHL* mutation in exon 1, and a *562C→G (L188V) VHL* mutation. The latter mutation has been previously described in a compound heterozygote of Anglo-US American Indian background who also carried the Chuvash *598 C→T VHL* mutation. Patients #2-4 were homozygous for the Chuvash *598 C→T* (*R200W*) *VHL* mutation. Patients #2 and #3 were US Caucasian brothers. Patient #4, a 17-year old US Caucasian unrelated to the others, also had congenital polycythemia. All three were polycythemic shortly after birth and were on a therapeutic phlebotomy program to maintain their hematocrit below 45%. They had the thrombotic complications depicted in Table 1. Patient #2 died at the age of 41 years, underscoring the thrombophilic nature of the Chuvash *598 C→T VHL* mutation.[7] Patient #4 had recurrent deep vein thrombosis with pulmonary embolism and mesenteric thrombosis at the age of 12 years and has been on

Table 1. Phenotypes of congenital polycythemia patients with *VHL* gene mutations.

Patients (Sex)	*Age (Years)*	*VHL Mutations*	*Serum Epo Level*	*Other clinical signs and symptoms*
1 (F)	33	235 C→T (R79C)* 562 C→G (L188V) Compound heterozygote	high	Asymptomatic
2 (M)	38	598 C→T (R200W) Homozygote	high	Portal and mesenteric vein thrombosis
3 (M)	41	598 C→T (R200W) Homozygote	high	Superficial venous thrombosis
4 (F)	17	598 C→T (R200W) Homozygote	high	Deep vein thrombosis pulmonary embolism
5 (F)	18	523 A→G (Y175C)* Heterozygote	normal	Ataxia telangiectasia

**Mutations were not detected in 100 US Caucasian chromosomes by Mfe1 restriction enzyme digestion and not found in 100 Portuguese chromosomes.*

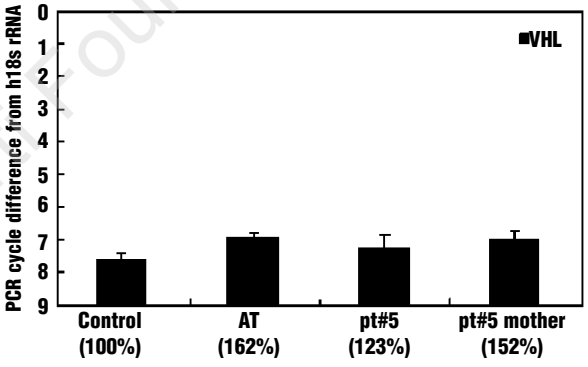

Figure 1. Quantification of VHL mRNA expression in patient #5 and control. VHL mRNA expression of patient #6 was compared to that of 4 normal controls, 3 patients with ataxia-telangiectasia (AT), and the mother of patient #5. The mean values of cycle differences from the h18S rRNA in real-time polymerase chain reactions (PCR) are shown with their standard deviations calculated from three independent experiments. Fewer cycles reflect a higher amount of mRNA. Each independent experiment contained mRNA from the four controls and three AT patients. The real-time PCR were run in duplicates. For each mRNA, the level is also expressed as a percentage of the control values. No significant difference was found between samples and controls ($p > 0.05$ calculated by one-way ANOVA together with Tukey's test).

chronic warfarin therapy. All three patients were investigated for thrombophilia and no underlying cause was found. The *VHL* core haplotype that has been observed in CP patients,[8] defined by six single nucleotide polymorphisms that span the 137.5 kb of the *VHL* gene, was found in all these three patients.

Patient #5 is a Portuguese girl with a normal P_{50}, normal Epo level and no evidence of cardiac, renal, brain, or adrenal pathology who has polycythemia and ataxia-telangiectasia (A-T). She is heterozygous for a novel *523 A→G (Y175C) VHL* mutation. This mutation is not in the Universal VHL-mutation Database (*www.umd.necker.fra*); however, a mutation in codon 175 was reported in a Spanish patient with pheochromocytoma; no nucleotide details were cited and no further details are available.[9] The parents of our patient are hematologically normal and there was no history of consanguinity. The *VHL* mutation was inherited from her father. The *VHL* gene of her mother was screened for aberrant mRNA transcripts by reverse transcription polymerase chain reactions and the exons and exon/intron boundaries were sequenced in both orientations from genomic DNA, and no mutation was found. A *VHL* null allele (or deletion) in the maternal gene was ruled out since equal proportions of wild-type and mutated nucleotides at 523 (A and G) were found in *VHL* cDNA. We have also considered that the polycythemic phenotype observed in A-T patient #5 may have been caused by a decreased amount of *VHL* mRNA transcript, perhaps caused by nonsense-mediated decay (NMD) secondary to the ATM defect, since the phosphorylation of NMD protein Upf1, a smg2 homolog, is reported to be ATM-dependent.[10] However, as we show in Figure 1, this possibility was ruled out. In summary, we conclude that defects in both alleles of the *VHL* gene may represent the most frequent inherited genetic polycythemic defect; we report two novel *VHL* mutations associated with polycythemia. The molecular biology of the polycythemic patients with a single mutated *VHL* allele remains obscure.

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Funding: this work was supported by grants R01HL66333-04 and R01HL5007-11 NHLBI from the National Institute of Health.

Acknowledgments: we are indebted to our clinical collaborators who referred the patients, provided the clinical material, and performed some of the studies: Drs. Stephen Moll, Wes Lee, L. Brown, and Yves Pastore.

Key words: congenital polycythemia, VHL mutations, ataxia-telangiectasia.

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Sustained response to interferon α-2a in thalassemic patients with chronic hepatitis C. A prospective 8-year follow-up study

Eighty-nine thalassemic patients with chronic hepatitis C were treated with interferon α-2a for 12 months and followed up for 8 years. Interferon induced sustained virologic and biochemical response in 45% of participants and histologic improvement in 50% of patients who had paired liver biopsies. Splenectomy was the only independent predictor of an unfavorable outcome.

haematologica 2005; 90:129-131
http://www.haematologica.org/journal/2005/1/129

Several studies on chronic hepatitis C in thalassemic patients have shown response rates to interferon higher than those in non-thalassemics.[1,2,3-7] These somewhat surprising findings are based on small number of patients with no long-term follow up. Herein, we present the results of a prospective 8-year follow-up study on the efficacy and safety of interferon α-2a in the treatment of chronic hepatitis C in 89 patients with β-thalassemia major.

An open prospective study was conducted from December 1994 through December 1995 to determine the efficacy of interferon α-2a in the treatment of chronic hepatitis C virus (HCV) infection in patients with β-thalassemia major. The participants were recruited from a cohort of 367 thalassemic patients who are followed up at our institution. Eligible patients were those who had detectable HCV RNA in serum, histologic findings consistent with chronic hepatitis, and elevated values of serum alanine aminotransferase. Patients with cardiovascular, endocrine, renal and autoimmune diseases or cirrhosis were excluded from the study as were those who had received prior treatment against hepatitis C. All patients were seronegative for human immunodeficiency virus and immune against hepatitis B virus. The participants were given 3MU of interferon α-2a (Roferon, Roche) subcutaneously thrice weekly for 52 weeks.

At study entry, all participants underwent a physical