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## To the editor:

### Effect of congenital upregulation of hypoxia inducible factors on percentage of fetal hemoglobin in the blood

Hypoxia inducible factor (HIF)-1 and HIF-2 are transcription factors that play a major role in cellular responses to hypoxia. Levels of the α subunits of HIFs are constantly degraded under normoxia by the proteasome through an interaction with their negative regulator, von Hippel-Lindau (VHL) protein.[1] Proline hydroxylation of HIF-α by prolyl hydroxylase domain enzymes is required for the interaction of HIF-α with VHL protein.[2] During hypoxia, this process is inhibited, leading to an accumulation of HIFs as HIF-α subunits translocate to the nucleus and interact with HIF-1β to form heterodimers. These heterodimeric transcription factors bind to hypoxia response elements leading to altered expression of genes, such as erythropoietin, that mediate the response to hypoxia.[3] Fetal hemoglobin is induced in human erythroid progenitors cultured under hypoxia[4] or exposed to prolyl hydroxylase inhibitors.[5] Fetal hemoglobin is also induced in red blood cells and reticulocytes of baboons exposed to a prolyl hydroxylase inhibitor[5] and humans exposed to high altitude for a limited period of time.[6] An increase in the actual percentage of fetal hemoglobin in the blood of the baboons given a prolyl hydroxylase inhibitor and the humans exposed to high altitude was not observed, but this may have been due to the small number of subjects in these studies or the limited duration of exposure to the experimental condition.

Chuvash polycythemia is an autosomal recessive congenital disorder characterized by a homozygous 598C>T mutation in the *VHL* gene, resulting in an R200W amino acid change in the VHL protein.[7] It is characterized by augmented HIF-1 and HIF-2 levels during normoxia and altered expression of erythropoietin and a number of other genes.[7-9] To determine if the percentage of fetal hemoglobin in the peripheral blood is increased in humans with chronically increased expression of HIFs, we performed high performance liquid chromatography (HPLC) hemoglobin fractionation in a cohort of 51 *VHL*$^{R200W}$ homozygotes, 28 *VHL*$^{R200W}$ heterozygotes, and 108 control subjects. The institutional review board of Howard University approved the study and the participants provided written informed consent. Individuals with a diagnosis of familial polycythemia or controls without such a diagnosis were studied in Chuvashia, Russia. Genotyping for the *VHL*$^{R200W}$ mutation was performed as previously described.[8] Hemoglobin F levels were determined by HPLC using the *ultra*$^2$-variant system (Trinity Biotech USA, Jamestown, NY). Samples were processed using the high-resolution method, which requires close to 11 minutes of run time per sample, allowing for higher sensitivity and specificity in detection. Serum erythropoietin concentrations were measured with an enzyme immunoassay in a little more than half of the subjects (R&D Systems, Minneapolis, MN) (reference range 3.3 to 16.6 IU/L).

Hemoglobin F was undetectable in most samples, regardless of *VHL* genotype, and there was no difference according to genotype in the proportion with detectable hemoglobin F: 7.8% of *VHL*$^{R200W}$ homozygotes had detectable hemoglobin F compared with 7.1% of *VHL*$^{R200W}$ heterozygotes and 8.3% of *VHL* wild-type subjects ($P > .9$) (Table 1). In no subject was the hemoglobin F percent greater than 1%. At the same time, the median serum erythropoietin concentration was more than fourfold higher in the *VHL*$^{R200W}$ homozygotes compared with the *VHL*$^{R200W}$ heterozygotes and *VHL* wild-type subjects ($P < .0001$).

We conclude that chronically increased levels of HIF-1 and HIF-2 to the degree observed in Chuvash polycythemia are not associated with an increase in hemoglobin F percent as determined by HPLC hemoglobin fractionation, despite an increase in the serum erythropoietin concentration.

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**Table 1. Proportions with detectable hemoglobin F and concentrations of erythropoietin in serum according to *VHL* genotype**

| | N | *VHL* WT | N | *VHL*$^{R200W}$ heterozygotes | N | *VHL*$^{R200W}$ homozygotes | P |
|---|---|---|---|---|---|---|---|
| Detectable hemoglobin F, no (%) | 108 | 9 (8.3%) | 28 | 2 (7.1%) | 51 | 4 (7.8%) | .98 |
| Erythropoietin, median (interquartile range) in U/L | 41 | 9.0 (7.8-13.7) | 25 | 9.4 (6.8-13.0) | 37 | 42.0 (24.8-100.2) | <.0001 |

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