Congenital disorder of oxygen-sensing: association of the homozygous Chuvash polycythemia VHL mutation with thrombosis and vascular abnormalities but not tumors

Short title for running head: CP VHL Mutation, Thrombosis and Vascular Abnormalities

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This work was supported in part by NIH research grant No. UH1-HL03679-05 from the National Heart, Lung and Blood Institute and the Office of Research on Minority Health, by Howard University General Clinical Research Center Grant No. MO1-RR10284, and NIH grant nos. R01HL66333-01 (JTP&VRG) and R01HL5007-09 (JTP).

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Word counts: abstract- 198   text- 4994
Scientific heading: Red Cells
Abstract

Adaptation to hypoxia is critical for survival and regulates multiple processes including erythropoiesis and vasculogenesis. Chuvash polycythemia is a hypoxia-sensing disorder characterized by homozygous mutation (598C>T) of von Hippel Lindau gene (VHL), a negative regulator of hypoxia-sensing. While endemic to the Chuvash population of Russia, this mutation occurs worldwide and originates from a single ancient event. That \( VHL598C>T \) homozygosity causes elevated normoxic levels of the transcription factor hypoxia inducible factor-1α (HIF-1α), serum erythropoietin and hemoglobin is known, but the disease phenotype has not been documented in a controlled manner. In this matched cohort study, \( VHL598C>T \) homozygosity was associated with vertebral hemangiomas, varicose veins, lower blood pressures and elevated serum vascular endothelial growth factor (VEGF) concentrations (P<0.0005), as well as premature mortality related to cerebral vascular events and peripheral thrombosis. Spinocerebellar hemangioblastomas, renal carcinomas, and pheochromocytomas typical of classical VHL were not found, suggesting that overexpression of HIF-1α and VEGF is not sufficient for tumorigenesis. While hemoglobin-adjusted serum erythropoietin concentrations were approximately 10-fold higher in \( VHL598C>T \) homozygotes than controls, erythropoietin response to hypoxia was identical. Thus, Chuvash polycythemia is a distinct VHL syndrome manifested by thrombosis, vascular abnormalities and intact hypoxic regulation despite increased basal expression of hypoxia-regulated genes.
**Introduction**

Chuvash polycythemia, the first hereditary condition of augmented hypoxia-sensing to be recognized, is an autosomal recessive disorder with increased serum erythropoietin levels and hemoglobin concentrations in normoxia.\(^1\)\(^-\)\(^5\) While congenital polycythemias are rare worldwide, hundreds of patients with Chuvash polycythemia are found in the Chuvash population of central Russia. We recently identified the mutation for Chuvash polycythemia to be \(598C>T\) in the von Hippel-Lindau gene (\(VHL\)) on chromosome 3p25.\(^4\) This mutation was subsequently found in persons of Caucasian, African-American and Pakistani/Bangladeshi ethnicities.\(^6\)\(^-\)\(^8\) Haplotype analysis indicates that the mutation originated from a single ancient event.\(^9\)

\(VHL\) protein, the recognition component of an E3 ubiquitin-protein ligase complex, mediates proteosomal degradation of the \(\alpha\) subunit of hypoxia inducible factor 1 (HIF-1) under normoxic conditions, whereas HIF-1\(\alpha\) is stable during hypoxia.\(^10\) HIF-1 is the principal transcriptional activator in hypoxic cells, promoting or enhancing transcription of erythropoietin and a number of other genes.\(^11\) Patients with Chuvash polycythemia have increased cellular levels of HIF-1\(\alpha\) during normoxia, and this accounts for the increased concentrations of serum erythropoietin and of hemoglobin\(^5\).

In classic VHL tumor predisposition syndrome, heterozygous germline mutations of \(VHL\) are associated with the development of renal clear cell carcinoma, pheochromocytoma, pancreatic neuroendocrine tumors, central nervous system hemangioblastoma, and other vascular tumors. Tumors develop from cells that acquire a somatic mutation of the unaffected \(VHL\) gene in addition to the germline mutation on the other allele. \(VHL\) codes for 213 amino acids, and over
130 distinct intragenic germline mutations associated with tumor predisposition have been identified, virtually all 5’ to the 598 position mutated in Chuvash polycythemia.\textsuperscript{12,13} Polycythemia is not a common manifestation of VHL syndrome, although increased hemoglobin concentrations can sometimes occur because of tumor production of erythropoietin.

Chuvash polycythemia differs from variants of VHL syndrome described thus far by the autosomal recessive rather than dominant pattern of inheritance, the relatively mild effect of the $598C>T$ mutation on the association of VHL with HIF-1\textsuperscript{\alpha}, and polycythemia as the presenting clinical manifestation.\textsuperscript{5} The natural history of Chuvash polycythemia has not been documented in a controlled study, and whether, as with other VHL mutations, there is an increased incidence of tumors is not known. The objective of this matched cohort study of patients with Chuvash polycythemia was to confirm our uncontrolled observation of premature mortality\textsuperscript{1-3} and to characterize the clinical effects of this inherited hypoxia-sensing defect.

**Methods**

**Study participants.** We conducted a retrospective study of mortality and a cross sectional study of morbidity from 2001 to 2003 in Chuvashia, Russia with patients diagnosed to have Chuvash polycythemia before 1977 by Dr. Lydia A. Polykova, one of the authors of this report.\textsuperscript{1,2} The median age at diagnosis of Chuvash polycythemia was 16 years (interquartile range of six to 22 years), and patients typically presented with plethora, headache and fatigue.\textsuperscript{1,2} We were able to visit or obtain information regarding 96 of 103 original patients. Two additional cohorts were studied- 65 spouses and 94 community members matched for age, sex and village of birth who were identified through the regional Zapis Actov Grazhdanskovo Sostoyaniya (ZAGS; Registry
of Citizen Status Events), a bureau that maintains records of births, deaths, and marriages. The Investigational Review Board of Howard University approved the investigations. Information was obtained from the research participant and/or medical records, death certificates, relatives, and neighbors. We recorded demographic and clinical characteristics and causes of death (Table 1). If the participant was living and available, we obtained informed consent, performed a medical history and physical examination and collected a venous blood sample.

**Blood samples.** Complete blood counts were performed with an automated method and genomic DNA was isolated from frozen Buffy coats within seven months of collection. Serum concentrations of erythropoietin were performed using ELISA on an Immulite instrument (Diagnostic Products Corp., Randolph, NJ.), of VEGF-165 by an enzyme immunometric assay (Assay Design, Inc., Ann Arbor, MI), of total PAI-1 using ELISA (HYPEN BioMed, Andrésy, France), and of transferrin receptor and ferritin by ELISA (Ramco Laboratories, Inc., Stafford, TX). Serum concentrations of the stable nitric oxide breakdown products, nitrite and nitrate (NO₃⁻), were assayed based on the conversion of nitrate to nitrite by the enzyme, nitrate reductase (Assay Designs, Inc., Ann Arbor, MI). Expected plasma ranges provided by the manufacturers are 5-35 IU/L for erythropoietin, 4-43 ng/ml for PAI-1, 'around 26 pg/ml' for VEGF, 2.9-8.3 mg/L for transferrin receptor, and 20-300 µg/L for ferritin. Expected ranges for nitrite and nitrate were not provided.

**PCR analysis for the VHL598C>T mutation.** Genomic DNA was isolated using a QIAGEN column (QIAGEN Inc, Valencia, CA) and PCR reactions were performed in 50 µl volumes containing 20 mM Tris-HCl pH 8.4, 50 mM KCl, 1.5 mM MgCl₂, 100 µM dNTP, 300 nM
primers, and 2.5 U/reaction Taq DNA polymerase (Life Technologies, Grand Island, NY). The following primers were used for amplification of \textit{VHL} exon 3: \textit{VHL}3F 5’-CCTTGTACTGAGACCCTAG, \textit{VHL}3R 5’-GCTGAGATGAAACAGTGTA. Ten µL of PCR product were incubated with 5 U of \textit{Fnu4HI} (New England Biolabs Inc, Beverly, MA) for 2 hours to detect the mutation. The \textit{VHL598C>T} mutation abolishes restriction sites for \textit{Fnu4HI} resulting in an uncut 296 base pair band detected on 1.2% agarose gel.

**Imaging and ophthalmologic studies in Chuvash polycythemia patients and control patients.** Computed tomography (CT) of the abdomen and magnetic resonance imaging (MRI) of the spine were performed in 33 patients with Chuvash polycythemia (32 of them documented to be \textit{VHL598C>T} homozygotes and one not tested) and 34 Chuvash patients without the diagnosis of Chuvash polycythemia. These patients without the diagnosis of polycythemia were in addition to the matched cohorts described above. MRI of the brain and ophthalmologic studies were performed in 33 patients with Chuvash polycythemia but not the patients without the diagnosis of Chuvash polycythemia. CT was performed through the abdomen with 5mm sections using helical mode. After obtaining preliminary non-enhanced scans, approximately 120cc of non-ionic iodinated contrast agent was injected and arterial and venous phase images were obtained. Routine clinical imaging of the brain and cervical and thoracic spine (including the entire spinal cord) was performed on a 1.5 T MRI system (GE Medical Systems, Milwaukee, WI) using T2 weighting and T1 weighting before and after intravenous injection of a Gadolinium chelate contrast agent. Indirect ophthalmoscopy and fluorescein angiography were performed by an ophthalmologist in Cheboksary, Russia.
Statistical analysis. Mortality was assessed with Kaplan-Meier analysis, the log rank test and Cox proportional hazards models. Clinical and laboratory measurements that were continuous variables were examined with linear regression, Spearman correlation, and or analysis of variance models that adjusted for important covariates. Variables that followed a skewed distribution were log transformed. Because serum concentrations of PAI-1 and VEGF are influenced by release by platelets,\textsuperscript{14,15} we adjusted for peripheral blood platelet counts in the analysis of variance models. Proportions were examined with the Pearson Chi-square test or the Fisher exact test. Logistic regression was used for certain statistical analyses.

Results

Demographic features of the patients with Chuvash polycythemia and the matched cohorts (Table 1). There were approximately equal numbers of men and women among the patients with Chuvash polycythemia, supporting the validity of using spouses as a matched cohort in addition to matched community members. More patients with Chuvash polycythemia died during the follow-up period encompassed by this study than spouses and community members. Histories of smoking and of diabetes mellitus were not over-represented among the patients with Chuvash polycythemia. Not shown in Table 1 are observations made in surviving participants that we examined (43 patients with Chuvash polycythemia, 46 spouses, and 49 community members) suggesting that obesity and hypertension were not over represented in the polycythemia cohort. Obesity, defined as body mass index $\geq 30$ kg/M$^2$, was found in 2.4\% of surviving patients with Chuvash polycythemia compared to 13.3\% of spouses and 12.2\% of community members. Elevated blood pressure, defined as systolic pressure $>140$ mm Hg and/or diastolic pressure $>90$
mm Hg, was found in 17.1% of surviving patients with Chuvash polycythemia compared to 47.8% of spouses and 22.9% of matched community members.

**Treatment of patients with Chuvash polycythemia.** Of the 96 patients with Chuvash polycythemia (Table 1), we were able to obtain information on treatment for 87 (91%). Seventy-eight percent of these had been treated with some degree of phlebotomy. The median age of starting phlebotomy therapy was 23 years (range of 3 to 48 years). The median intensity of phlebotomy therapy was 1.4 phlebotomies per year (<1 to 5 per year). The median volume of blood removed per phlebotomy was 500 ml (250 to 750 ml).

Among patients from whom we drew blood samples as part of the present study, the median hemoglobin concentration was 15.2 g/dL (range of 9.9 to 23.8 g/dL) in eight participants who averaged two or more phlebotomies per year compared to 18.0 g/dL (12.7 to 25.4 g/dL) in 34 participants who averaged less than two phlebotomies per year (P = 0.2). The median hemoglobin concentration was 19.0 g/dL (13.9 to 25.4 g/dL) in 13 patients who gave a history of thrombosis (see below) while it was 16.6 g/dL (9.9 to 23.8 g/dL) in 33 participants who did not (P = 0.064). The median hemoglobin concentration was 18.0 g/dL (12.7 to 23.8 g/dL) in 19 patients who had MRI or CT scan evidence of thrombosis (see below) while it was 16.6 g/dL (9.9 to 21.6 g/dL) in 13 participants who did not (P = 0.2).

Forms of treatment other than phlebotomy included aspirin in 39% of the patients and busulfan in 17%. In a logistic regression model that examined the relationship of thrombosis history to a history of treatment with aspirin and whether or not the patient had averaged at least two
phlebotomies per year, aspirin was associated with a 2.4-fold increase in the odds of thrombosis (95 confidence interval of 0.7 to 7.7; \( P = 0.15 \)) and phlebotomy with a 5.6-fold decrease (0.7 to 47.6; \( P = 0.12 \)). Busulfan had been administered between the years of 1959 and 1971, before it was realized that Chuvash polycythemia was a distinct condition from polycythemia vera. The median age at the time of treatment with busulfan was 20 years (range of 12 to 46 years). One patient was treated with radioactive phosphorous in addition to busulfan.

**Mortality.** Cerebral vascular events were especially common as a cause of death among patients with Chuvash polycythemia (Table 1). Almost half of the cerebral vascular events in Chuvash polycythemia patients were described as hemorrhagic, but this characterization may not have been confirmed radiographically or anatomically. The median age at death from a cerebral vascular event was 42 years (range of 26 to 70 years) in 11 patients with Chuvash polycythemia and 70 years (58 to 81 years) in three control participants. Four of the patients but none of the spouse and community cohort members died from mesenteric thrombosis, and the median age of death was 49 years (27 to 59 years).

Among 65 patient-spouse pairs, estimated survival to age 65 years was 25% for patients with Chuvash polycythemia versus 69% for spouses (\( P = 0.002 \)) (Figure 1a). Among all 94 patient-community member pairs, estimated survival to age 65 years was 31% for patients with Chuvash polycythemia versus 54% for community members (\( P = 0.17 \)) (Figure 1b). Death from pneumonia, measles and varicella was 16.0% by age three years in community members, reflecting high infant and childhood mortality in Chuvashia in the mid-twentieth century, and this high early mortality in community members appeared to bias the analysis in the 94 patient-
community member pairs. Therefore, we also analyzed mortality for 76 patient-community member pairs in which the community member survived to at least age 16 years, the median age of diagnosis of Chuvash polycythemia, and found that estimated survival to age 65 years was 29% for patients with Chuvash polycythemia versus 64% for community members \( (P < 0.0005) \) (Figure 1c). After adjustments for histories of alcohol use and smoking, the hazards ratio of death for Chuvash polycythemia patients was 4.3 (95% confidence interval of 2.0 to 9.3) in the 65 patient-spouse pairs and 3.8 (1.8 to 7.8) in the 76 patient-community member pairs. The adjusted hazards ratio of death from cerebral vascular events for Chuvash polycythemia patients was 15.6 (1.5 to 160.9; \( P = 0.021 \)) in the patient-spouse pairs and 17.3 (1.3 to 239.6; \( P = 0.033 \)) in the patient-community member pairs. The hazards ratio of death from peripheral thrombosis was also significantly higher for the Chuvash polycythemia patients in the analysis of the 65 patient-spouse pairs \( (P = 0.038) \) but not the patient-community member pairs. The hazards ratio for other causes of death (Table 1) did not reach statistical significance. In particular, we found no evidence of increased deaths from malignancy among the patients with Chuvash polycythemia although some had received treatment with the alkylating agent, busulfan, an agent with leukemia- and other cancer-inducing properties.\(^{16}\)

Among the 96 patients with Chuvash polycythemia, univariate analyses indicated that male sex was associated with a 5.3-fold increase in the hazards ratio of death \( (P < 0.0005) \), history of smoking with a 2.3-fold increase \( (P = 0.013) \), history of alcohol consumption with a 2.5-fold increase \( (P = 0.010) \), and receiving some degree of phlebotomy therapy with a 1.6-fold decrease \( (P = 0.2) \). In a multivariate analysis that included all of these variables, histories of smoking and alcohol consumption no longer had a significant influence on the hazards ratio of death \( (P > 0.6) \),
but male sex was associated with a 4.6-fold increase in the ratio (95% confidence interval of 1.6 to 12.9; P = 0.004) and history of phlebotomy therapy with a 1.7-fold decrease (0.8 to 3.7; P = 0.16). In similar multivariate analyses, only male sex had a trend of increasing the hazards ratio of death from cerebral vascular events (P = 0.16) and from peripheral thrombosis (P = 0.001).

Clinical findings in the living research participants according to VHL genotype. PCR analysis for the \textit{VHL598C>T} mutation revealed a perfect genotype-phenotype correlation for clinically diagnosed Chuvash polycythemia, with all 43 patients and none of 86 spouses or community members genotyped proving to be homozygotes for the mutation. Seven spouses (16%) and two community members (5%) were heterozygotes. Forty-one (95%) of the \textit{VHL598C>T} homozygotes gave a history of phlebotomy therapy, often for cosmetic reasons, with a frequency ranging from 0.3 to 5 phlebotomies per year and a median of 1.1 per year. In addition to higher hemoglobin concentrations, homozygotes for \textit{VHL598C>T} had more frequent thromboses by history, lower systolic and diastolic blood pressures, more venous varicosities, and lower white blood cell and platelet counts compared to unaffected research participants (Table 2; P < 0.0005). While there were no significant differences between unaffected participants and the small number of \textit{VHL598C>T} heterozygotes for many of these clinical observations, adjusted systolic and diastolic blood pressures were significantly lower in the heterozygotes than the unaffected participants and paralleled the findings for the homozygotes. We found no significant differences by genotype in the reported history of cancer, which was low, and in adjusted serum ferritin concentrations. Among the \textit{VHL 598C>T} homozygotes, we did not observe significant differences in the variables listed in Table 2 according to whether or not the participants were treated with a phlebotomy intensity averaging two or more per year.
Circulating concentrations of proteins or metabolites of proteins encoded by HIF-1-regulated genes. Adjusted mean serum concentrations of VEGF and total PAI-1 were significantly higher in VHL598C>T homozygotes than unaffected participants (P < 0.0005), but concentrations of the end products of nitric oxide breakdown, nitrite/nitrate, were not (Figure 2). Consistent with our previous studies,3-5 adjusted serum levels of erythropoietin and transferrin receptor were also significantly higher in homozygotes. Only adjusted serum total PAI-1 levels differed significantly between VHL598C>T heterozygotes and unaffected participants (P = 0.032).

In 41 VHL598C>T homozygotes, Spearman correlation revealed significant inverse correlations of serum erythropoietin concentration with number of months after last phlebotomy (R = -0.295, P = <0.05) and hemoglobin concentration (R = -0.519, P = <0.005). In a linear regression analysis that examined the relationship of serum erythropoietin concentration with both hemoglobin concentration and phlebotomy history in the same model, the significant negative correlation persisted with hemoglobin concentration (P = 0.001) but not with time since last phlebotomy (P = 0.5).

We contrasted the relationship of serum erythropoietin concentration with hemoglobin concentration and the relationship of serum transferrin receptor concentration with serum ferritin concentration among 41 VHL598C>T homozygotes and 70 unaffected participants (Figure 3). Although, the hemoglobin-adjusted erythropoietin concentration was approximately 10-fold higher in VHL598C>T homozygotes than unaffected participants (Figure 2d), the response of
erythropoietin to hypoxia, as indicated by the slope of the regression line between log erythropoietin concentration and hemoglobin concentration, was identical between these two groups (-0.007 log erythropoietin units per 1 g/dL hemoglobin; Figure 3a). Similarly, although the serum ferritin-adjusted transferrin receptor concentration was approximately three-fold higher in \textit{VHL598C>T} homozygotes than unaffected participants (Figure 2e), the response of transferrin receptor to changing iron status, as indicated by the slope of the regression line between log transferrin receptor concentration and log ferritin concentration, was almost identical between these two groups (-0.154 log transferrin receptor unit per log ferritin unit in homozygotes versus -0.158 in unaffected subjects; Figure 3b).

Further analyses among the 129 research participants included in Table 2 revealed the following. In linear regression models that adjusted for VHL mutation status, age, body mass index and history of smoking, a 10-fold increase in serum VEGF concentration was associated with an estimated 24 mm Hg decrease in systolic blood pressure (95% confidence interval of 4 to 44 mm Hg; \(P = 0.021\)) and an estimated 24 mm Hg decrease in pulse pressure (6 to 42 mm Hg; \(P = 0.008\)), but there was no significant correlation between diastolic blood pressure and serum VEGF concentration. Serum nitrite/nitrate concentrations did not correlate significantly with blood pressure. In an analysis of variance model that adjusted for VHL mutation status, there was no significant difference in serum VEGF concentrations according to the presence or absence of varicose veins on physical examination. However, among the subgroup of \textit{VHL598C>T} homozygotes, the geometric mean (SD range) serum VEGF concentration 113 (67 to 191) pg/ml in patients with varicose veins was significantly higher than the concentration of 81 (60 to 111) pg/ml in patients without varicose veins (\(P = 0.018\)). No significant correlation of
serum PAI-1 concentrations with a history of thrombosis was found in analysis of variance models.

**Imaging and ophthalmologic studies in patients with Chuvash polycythemia.** We invited 44 living Chuvash polycythemia patients, 43 of them documented to be *VHL*598C>T homozygotes, to participate in imaging and ophthalmologic studies and 33 agreed, giving written informed consent. We also recorded the results of CT scans of the abdomen and MRI of the spinal cord in 34 Chuvash patients who did not have the diagnosis of Chuvash polycythemia; these patients were in addition to the matched community members described above. The patients were not acutely ill and not hospitalized at the time of the studies. The results are summarized in Table 3.

*Studies performed only in patients with Chuvash polycythemia.* Magnetic resonance imaging of the brain of 33 patients with Chuvash polycythemia did not demonstrate cerebellar or supratentorial hemangioblastomas. Old infarcts or chronic ischemic lesions were identified in 14 (45.2%). Ophthalmologic examination in these patients showed no retinal hemangioblastomas. Serum PAI-1 concentrations did not differ significantly according to the presence or absence of cerebral ischemic lesions on MRI.

*Studies performed in both patients with Chuvash polycythemia and Chuvash patients without polycythemia.* MRI of the spine did not demonstrate direct or indirect evidence of spinal cord hemangioblastomas (i.e. enhancing tumor or associated cord edema). However, vertebral body hemangiomas were identified in 18 (54.5%) of 33 patients with Chuvash polycythemia compared to 7 (20.6%) of 34 Chuvash patients without polycythemia (*P* = 0.006). In a logistic regression model, both presence of Chuvash polycythemia (*P* = 0.002) and age (*P* = 0.004) were associated with significant increases in the odds of having vertebral hemangiomas. Diagnosis of Chuvash
polycythemia was associated with a 7.0-fold increase in the odds of finding vertebral hemangioma by MRI (95% confidence interval of 2.0 to 24.5) and an increase in age by 10 years was associated with a 3.1-fold increase in the odds (1.4 to 6.7). Among the patients with Chuvash polycythemia, serum VEGF concentrations were not higher in patients with vertebral hemangiomas. CT scanning of the abdomen revealed no significant differences in the incidence of cystic lesions of the kidney or liver between Chuvash patients with and without polycythemia. None of the patients with Chuvash polycythemia had findings suggestive of renal cell carcinoma or pheochromocytoma.

Discussion
Our results indicate that Chuvash polycythemia is a unique VHL syndrome characterized by homozygous germline mutation of \textit{VHL}, increased mortality due to cerebral vascular events and peripheral thrombosis, distinct vascular abnormalities, intact hypoxia sensing despite increased systemic expression in normoxia of a broad range of HIF-1-regulated genes, and absence of a predisposition to develop tumors or malignancy. Limitations to our study include that 1) data on mortality was obtained retrospectively from a variety of sources; 2) historical findings of the living participants were subject to recall bias; and 3) the sample size is rather small for an epidemiological study. Nevertheless, that we used a defined set of subjects diagnosed before 1977 as patients, studied the patients and matched cohorts in a narrow time frame, and used both a spouse cohort and a rigorously-matched community cohort to test study hypotheses strengthens the validity of our findings. Furthermore, the sample size is quite large for the study of a condition as rare as congenital polycythemia.
Premature mortality from cerebral vascular events and peripheral thrombosis. The peripheral and central nervous system thrombosis and the premature mortality we observed in Chuvash polycythemia have parallels with the complications of polycythemia vera, an acquired clonal disease due to a somatic hematopoietic stem cell mutation. A comparison of our follow-up of 96 Chuvash polycythemia patients with the follow-up of 1213 Italian polycythemia vera patients is instructive. Most patients in both cohorts received treatment, with the Chuvash polycythemia patients predominantly receiving phlebotomy therapy alone and the Italian polycythemia vera patients predominantly receiving myelosuppressive therapy; the degree of disease control for the Italian patients was not reported. The median age at diagnosis of Chuvash polycythemia was younger (16 years versus about 60 years), the period of follow-up was longer (over 23 years in all Chuvash polycythemia patients versus less than 20 years in 90% of polycythemia vera patients), overall mortality was higher (47% versus 18.5%), age- and-sex-standardized mortality rate was higher (3.8 times matched community members versus 1.7 times the general population) and proportion of deaths due to cerebral vascular events or peripheral thrombosis was higher (46.1% versus 21.9%) despite the younger age of the Chuvash polycythemia patients. It is also of note that other polycythemic conditions, such as those associated with erythropoietin receptor mutations, high altitude, hemoglobin mutants with high oxygen affinity, and 2,3-biphosphoglycerate deficiency, do not seem to have similar high rates of thrombotic complications, and transgenic mice with constitutive over-expression of human erythropoietin have hematocrits up to 85% with no thrombotic tendency. Therefore, Chuvash polycythemia may be a polycythemic condition with an unusually high tendency for thrombosis, but any firm conclusions in this regard require further study. That we failed to find a statistically significant beneficial effect of phlebotomy therapy on mortality and thrombotic complications...
suggests that the thrombotic tendency may not correlate with hematocrit and may therefore be caused by a factor or factors yet to be identified. Further study is also needed to definitively ascertain the potential benefit of phlebotomy therapy for this condition.

**Vascular abnormalities.** The increased prevalence of vertebral hemangiomas and varicose veins and the lower blood pressures that we observed in \(598C>T\) VHL homozygotes in the present study are in contrast to the absence of recognized increased occurrence of hemangiomas and varicose veins and to the presence of a tendency for hypertension in patients with polycythemia vera\(^{17,19}\) and primary familial and congenital polycythemias due to gain of erythropoietin function.\(^{22}\) The background prevalence of 21% for vertebral hemangiomas in 34 Chuvash patients without polycythemia in the present study, which can be compared to the prevalence of 11% found at autopsy elsewhere,\(^{23}\) supports the conclusion that the finding of this benign vascular malformation in 55% of the 33 Chuvash polycythemia patients imaged in this study represents a true disease association. The incidence of vertebral hemangiomas in classic VHL syndrome has not been documented. The lower blood pressures in Chuvash polycythemia patients in the present study are in contrast to the tendency for hypertension observed in humans exposed to chronic hypoxia\(^{24}\) or recombinant erythropoietin therapy,\(^{25}\) suggesting the hypotensive tendency is the effect of another HIF-1 target gene or genes than erythropoietin. Our observation that blood pressures are significantly lower in \(VHL598C>T\) heterozygotes than unaffected participants, if confirmed in future studies, points to a possible survival advantage for carriers of this mutation in protection from hypertension. Another intriguing possibility is that \(VHL598C>T\) heterozygosity might afford protection from preeclampsia,\(^{26}\) the leading cause of maternal and fetal mortality worldwide.
Serum levels of products of hypoxia-regulated genes. Our results provide further evidence that a broad range of hypoxia-regulated genes are up regulated under normoxic conditions in patients with Chuvash polycythemia.

Erythropoietin and transferrin receptor. The identical increase in serum erythropoietin concentration per unit decrease in hemoglobin concentration that we observed in \( VHL598C>T \) homozygotes and unaffected participants in the present study (Figure 3a), suggests that the hypoxia sensing mechanism functions normally in \( VHL598C>T \) homozygotes in a setting of marked basal up-regulation of HIF-1\( \alpha \)-regulated genes. Also, given that low intracellular iron concentration enhances HIF-1\( \alpha \) expression in a similar manner to hypoxia\(^{27} \) and that serum ferritin concentration reflects iron status,\(^ {28} \) the virtually identical increase in serum transferrin receptor concentration per unit decrease in serum ferritin concentration in \( VHL598C>T \) homozygotes and unaffected participants (Figure 3b) suggests that both the cellular iron-sensing and hypoxia-sensing mechanisms function normally in this setting.

VEGF. The serum VEGF levels we observed in \( VHL598C>T \) homozygotes in the present study overlap those reported in patients with classic VHL syndrome in two studies,\(^ {29,30} \) but our results contrast with the lack of finding significantly higher levels in patients with classic VHL syndrome compared to controls in one study.\(^ {29} \) Other studies have shown that parenteral administration of VEGF lowers systemic blood pressure in experimental animals\(^ {31} \) and humans.\(^ {32} \) In our analyses that adjusted for \( VHL598C>T \) mutation status, serum VEGF concentrations demonstrated a significant negative correlation with systolic blood pressure but not diastolic blood pressure. While it is possible that that increased expression of VEGF contributes to lower blood pressure in Chuvash polycythemia patients, a number of other genes up regulated by HIF-
whose products we did not measure, such as α1B-adrenergic receptor, adrenomedullin, endothelin-1 and heme oxygenase-1, could also plausibly contribute to blood pressure changes. Plasma VEGF levels have been reported to be increased in patients with varicose veins, and we observed an association between serum levels of VEGF and varicose veins among the VHL<sup>598C>T</sup> homozygotes but not the unaffected subjects in the present study. Although increased expression of VEGF or mutations of its receptor have been associated with hemangiomas in mice, and humans, we did not observe higher serum VEGF levels among the Chuvash polycythemia patients with vertebral hemangiomas compared to those without vertebral hemangiomas in this study.

**Nitrite/Nitrate.** Serum concentrations of nitrite/nitrate, the stable end products of nitric oxide metabolism, were not elevated in VHL<sup>598C>T</sup> homozygotes compared to unaffected subjects and did not correlate significantly with either systolic or diastolic pressure in the present study. Nitric oxide is formed from L-arginine by nitric oxide synthase and the endothelial form of nitric oxide synthase, rather than the hypoxia-regulated inducible form, seems to predominate in the vascular system.

**PAI-1.** Whether the increased circulating levels of total PAI-1 we observed in VHL<sup>598C>T</sup> homozygotes in this study might have contributed to thrombogenesis is not clear. PAI-1 is the primary inhibitor in plasma of activators of plasminogen. Transgenic mice with increased expression of PAI-1 suffer spontaneous thromboses, and an association between elevated PAI-1 levels and thrombosis in humans has been observed in some but not all studies. In the present study, we did not find higher serum total PAI-1 levels in the living Chuvash polycythemia patients according to a history of thrombosis or imaging evidence of thrombosis.
Absence of tumors of classic VHL syndrome. The failure to find VHL syndrome tumors in Chuvash polycythemia patients is striking, for all cells are homozygous for a mutation that leads to increased expression of HIF-1α and VEGF in normoxia. The development of hemangioblastoma and renal cell carcinoma associated with other heterozygous VHL mutations in the context of VHL tumor predisposition syndrome seems to be related to increased expression of HIF\textsuperscript{41,42} and possibly VEGF.\textsuperscript{43} Our findings are consistent with the concept that deregulation of HIF-1 and VEGF may not be sufficient to cause tumorigenesis in VHL syndrome.\textsuperscript{41} VHL protein has other substrates than HIF, some yet unidentified, that may have roles in the growth and behavior of cells,\textsuperscript{12,41,42} and interaction with these substrates might not be affected by the Chuvash mutation. Also, the \textit{VHL598C>T} mutation results in a relatively mild increase in HIF-1α expression,\textsuperscript{5} while more severe dysregulation may be necessary to promote tumorigenesis.

Implications of early mortality for \textit{VHL598C>T} homozygotes but high gene frequency in the Chuvash population. Our demonstration of decreased survival for \textit{VHL598C>T} homozygotes indicates possible negative selection pressure for Chuvash polycythemia, especially since early mortality begins during child-bearing age (Figure 1). In contrast, that this mutation has world-wide distribution, originated from a single founder a large number of generations past, and is found in various ethnicities\textsuperscript{9} suggests that there is no negative selection of the carrier state and possible survival advantage for heterozygotes. Considering the many generations since the occurrence of this mutation and the rarity of this disease outside the Chuvash ethnic isolate, any survival advantage is probably subtle. Whether the potential survival benefit of \textit{VHL598C>T} heterozygosity might be associated with up-regulation of iron absorption...
and erythropoiesis after blood loss, possible amelioration of pre-eclampsia, or other effects remains to be explored by future studies.
References


31. Li B, Ogasawara AK, Yang R, Wei W, He GW, Zioncheck TF, Bunting S, De Vos AM, Jin H. KDR (VEGF receptor 2) is the major mediator for the hypotensive effect of VEGF. Hypertension 2002;39:1095-100.


**Figure legends**

**Figure 1.** Kaplan-Meier survival curves for Chuvash polycythemia patients and spouses or community members matched for age, sex and place of birth. a. 65 Chuvash polycythemia patients and 65 spouses. b. 94 Chuvash polycythemia patients and 94 matched community members. There was high mortality for the community members in the first three years of life, while the median age of diagnosis of Chuvash polycythemia was 16 years. c. 76 Chuvash polycythemia patients and 76 matched community members who survived to age 16 years.

**Figure 2.** Adjusted mean ± SE concentrations of the products or metabolites of selected HIF-1-regulated genes according to *VHL*<sup>598C>T</sup> genotype in persons of Chuvash ethnicity. a. Serum vascular endothelial growth factor (VEGF)- analysis of variance (ANOVA) model with adjustment for peripheral blood platelet count. b. Serum nitrite/nitrate, stable end products of nitric oxide breakdown. c. Serum plasminogen activator inhibitor 1 (PAI-1)- ANOVA model with adjustment for peripheral blood platelet count. d. Serum erythropoietin (EPO)- ANOVA model with adjustment for sex and hemoglobin concentration. e. Serum transferrin receptor-ANOVA model with adjustment for serum ferritin concentration.

The numbers of subjects for each genotype category are as indicated in Table 2. The P value represents the significance level for the overall ANOVA and also for the comparison of unaffected participants with *VHL*<sup>598C>T</sup> homozygotes. Only for PAI-1 was there a significant difference between unaffected participants and *VHL*<sup>598C>T</sup> heterozygotes, and this was at the 0.03 significance level.
Figure 3. Responses to hypoxia and to iron status are intact in VHL598C>T homozygotes despite increased basal expression of hypoxia-regulated genes. a. The relationship of serum erythropoietin concentration to hemoglobin concentration in 41 VHL598C>T homozygotes and 70 unaffected participants, all of Chuvash ethnicity, is depicted. Regression lines and 95 percent confidence intervals are shown for each group. The dashed horizontal lines represent the lower (5 IU/L) and upper (35 IU/L) limits of the reference range for erythropoietin. The slope of the regression line is identical for each group (-0.007 log erythropoietin unit per 1 g/dL hemoglobin).

b. The relationship of serum transferrin receptor concentration to serum ferritin concentration in the same subjects is depicted. Regression lines and 95 percent confidence intervals are shown for each group. The dashed horizontal lines represent the lower (2.3 mg/L) and upper (8.9 mg/L) limits of the reference range for transferrin receptor. The slope of the regression line for the VHL598C>T homozygotes (-0.154 log transferrin receptor unit per log ferritin unit) is almost identical to the slope for unaffected subjects (-0.158).
Table 1. Demographic characteristics and causes of death of patients with Chuvash polycythemia and the matched cohorts (individuals who were traced by personal contact, medical records, civic records, and/or contact with family and community members)

<table>
<thead>
<tr>
<th>Demographic characteristics</th>
<th>Chuvash Polycythemia Patients</th>
<th>Spouses</th>
<th>Community Members</th>
<th>Community Members Surviving to Age 16 Years</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age in years at time of last contact or death; median (range)</td>
<td>44 (12-74)</td>
<td>46 (26-72)</td>
<td>44 (0.1–81)</td>
<td>46 (16-81)</td>
</tr>
<tr>
<td>Female sex; no. (%)</td>
<td>55 (57.3)</td>
<td>28 (43.1)</td>
<td>53 (56.4)</td>
<td>43 (56.6)</td>
</tr>
<tr>
<td>History of smoking; no. (%)</td>
<td>22 (23.7)</td>
<td>22 (36.1)</td>
<td>22 (25.0)</td>
<td>22 (32.4)</td>
</tr>
<tr>
<td>History of alcohol consumption; no. (%)</td>
<td>21 (22.6)</td>
<td>25 (41.0)</td>
<td>14 (15.9)</td>
<td>14 (20.6)</td>
</tr>
<tr>
<td>History of diabetes mellitus; no. (%)</td>
<td>1 (1.1)</td>
<td>2 (3.3)</td>
<td>1 (1.1)</td>
<td>1 (1.5)</td>
</tr>
<tr>
<td>Died during the follow up period; no. (%)</td>
<td>45 (46.9)</td>
<td>11 (16.9)</td>
<td>28 (29.8)</td>
<td>11 (14.5)</td>
</tr>
</tbody>
</table>

Causes of death; no.

<table>
<thead>
<tr>
<th></th>
<th>Chuvash Polycythemia Patients</th>
<th>Spouses</th>
<th>Community Members</th>
<th>Community Members Surviving to Age 16 Years</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cerebral vascular events</td>
<td>11</td>
<td>1</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Thrombotic events, non-cerebral</td>
<td>10</td>
<td>3</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Trauma</td>
<td>8</td>
<td>3</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Cardiac valvular disease</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Cancer</td>
<td>2</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Hemorrhage, non-cerebral</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Hypertensive crisis</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Pneumonia</td>
<td>0</td>
<td>1</td>
<td>9</td>
<td>1</td>
</tr>
<tr>
<td>Childhood diseases (measles; chicken pox)</td>
<td>0</td>
<td>0</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>Other</td>
<td>8</td>
<td>3</td>
<td>5</td>
<td>1</td>
</tr>
</tbody>
</table>

*Chuvash polycythemia- 5 hemorrhagic, 5 unspecified, 1 thrombotic; spouses- 1 hemorrhagic; community members- 2 unspecified.

*Chuvash polycythemia- 4 coronary artery; 4 mesenteric; 1 leg; 1 pulmonary; spouses- 2 coronary artery, 1 leg; community members- 4 coronary artery.

*Chuvash polycythemia- 1 lung cancer, 1 gastric cancer; community members- 1 gastric cancer.
Table 2. Clinical findings of study participants according to VHL genotype.

<table>
<thead>
<tr>
<th>VHL598C&gt;T homozygote (n = 40-43)</th>
<th>VHL598C&gt;T heterozygote (n = 8-9)</th>
<th>Unaffected (n = 70-77)</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Demographic Information</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (years; mean ± SE)</td>
<td>49 ± 1</td>
<td>45 ± 3</td>
<td>50 ± 2</td>
</tr>
<tr>
<td>Female sex (no. (%))</td>
<td>32 (74.4)</td>
<td>6 (66.7)</td>
<td>38 (49.4)</td>
</tr>
<tr>
<td><strong>Medical History</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Treated by phlebotomy (no. (%))</td>
<td>41 (95.3)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Headache (no. (%))</td>
<td>37 (86.0)</td>
<td>5 (62.5)</td>
<td>36 (46.8)</td>
</tr>
<tr>
<td>Dizziness (no. (%))</td>
<td>25 (58.1)</td>
<td>1 (12.5)</td>
<td>13 (16.9)</td>
</tr>
<tr>
<td>Dyspnea on exertion (no. (%))</td>
<td>24 (55.8)</td>
<td>1 (11.1)</td>
<td>11 (14.3)</td>
</tr>
<tr>
<td>Thrombosis (no. (%))</td>
<td>11 (25.6)</td>
<td>0 (0)</td>
<td>1 (1.3)</td>
</tr>
<tr>
<td>Bleeding (no. (%))</td>
<td>14 (32.6)</td>
<td>0 (0)</td>
<td>7 (9.1)</td>
</tr>
<tr>
<td>Peptic ulcer disease (no. (%))</td>
<td>13 (30.2)</td>
<td>0 (0)</td>
<td>9 (11.7)</td>
</tr>
<tr>
<td>Cancer (no. (%))</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>1 (1.3)</td>
</tr>
<tr>
<td><strong>Physical Examination</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weightb (kg; mean ± SE)</td>
<td>60.9 ± 1.9</td>
<td>69.2 ± 4.0</td>
<td>65.7 ± 1.4</td>
</tr>
<tr>
<td>Heightb (M, mean ± SE)</td>
<td>1.62 ± 1.05</td>
<td>1.60 ± 2.22</td>
<td>1.62 ± 0.77</td>
</tr>
<tr>
<td>Body mass indexb (kg/M²; mean ± SE)</td>
<td>23.2 ± 0.6</td>
<td>27.0 ± 1.4</td>
<td>24.9 ± 0.5</td>
</tr>
<tr>
<td>Obesity (body mass index ≥ 30 kg/M²)</td>
<td>1 (2.4)</td>
<td>1 (11.1)</td>
<td>9 (12.0)</td>
</tr>
<tr>
<td>Systolic BPc (mm Hg; mean ± SE)</td>
<td>120 ± 3</td>
<td>119 ± 6</td>
<td>133 ± 2</td>
</tr>
<tr>
<td>Diastolic BPc (mm Hg; mean ± SE)</td>
<td>79 ± 2</td>
<td>78 ± 5</td>
<td>87 ± 2</td>
</tr>
<tr>
<td>Plethora (no. (%))</td>
<td>33 (76.7)</td>
<td>1 (11.1)</td>
<td>9 (11.7)</td>
</tr>
<tr>
<td>Varicose veins (no. (%))</td>
<td>32 (74.4)</td>
<td>2 (22.2)</td>
<td>30 (39.0)</td>
</tr>
<tr>
<td>Edema (no. (%))</td>
<td>14 (32.6)</td>
<td>1 (11.1)</td>
<td>5 (6.6)</td>
</tr>
<tr>
<td>Clubbing (no. (%))</td>
<td>6 (14.0)</td>
<td>0 (0)</td>
<td>1 (1.3)</td>
</tr>
<tr>
<td>Splenomegaly (no. (%))</td>
<td>3 (7.0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td><strong>Laboratory Data</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hemoglobinb (g/dL; mean ± SE)</td>
<td>18.3 ± 0.3</td>
<td>13.3 ± 0.4</td>
<td>12.9 ± 0.2</td>
</tr>
<tr>
<td>Hematocritb (%; mean ± SE)</td>
<td>59.3 ± 0.9</td>
<td>41.3 ± 1.9</td>
<td>40.4 ± 0.7</td>
</tr>
<tr>
<td>White blood cells (no./µL; mean ± SE)</td>
<td>5,300 ± 200</td>
<td>7,900 ± 500</td>
<td>6,600 ± 200</td>
</tr>
<tr>
<td>Platelets (no/µL; mean ± SE)</td>
<td>167,000 ± 10,000</td>
<td>265,000 ± 22,000</td>
<td>238,000 ± 8,000</td>
</tr>
<tr>
<td>Ferritinb (µg/L; geometric mean and SE range)</td>
<td>67 (37-122)</td>
<td>20 (12-34)</td>
<td>30 (22-41)</td>
</tr>
</tbody>
</table>

*a*P value for comparison between VHL598C>T homozygotes and unaffected subjects by analysis of variance or by Pearson chi square. Only systolic blood pressure (P = 0.040), diastolic blood pressure (P = 0.044) and white blood cells (P = 0.033) differed significantly between heterozygotes and unaffected participants.

b Analysis of variance model in which weight is adjusted for age and sex.

c Analysis of variance models in which blood pressure is adjusted for pulse and body mass index.

d Analysis of variance model in which ferritin adjusted for age, sex and amount of blood removed by phlebotomy.
Table 3. Results of imaging studies of 33 patients with Chuvash polycythemia and 34 Chuvash patients without polycythemia.

<table>
<thead>
<tr>
<th></th>
<th>Chuvash polycythemia (N = 33)</th>
<th>Chuvash patients without polycythemia (N = 34)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female sex (no. (%))</td>
<td>23 (69.7)</td>
<td>22 (64.7)</td>
<td>0.8</td>
</tr>
<tr>
<td>Age (mean ± SD)</td>
<td>47 ± 9</td>
<td>48 ± 9</td>
<td>0.6</td>
</tr>
<tr>
<td><strong>MRI of brain</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cerebellar hemangioblastoma</td>
<td>0 (0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cerebellar venous angioma (no. (%))</td>
<td>1 (3.2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Quadrigeminal plate lipoma (no. (%))</td>
<td>1 (3.2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chiari I malformation</td>
<td>2 (6.5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cerebral ischemia or infarct (no. (%))</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Overall</td>
<td>14 (45.2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Small vessel ischemia</td>
<td>7 (22.6)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lacunar infarcts</td>
<td>7 (22.6)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Old frontal infarct</td>
<td>2 (6.5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bilateral watershed infarct</td>
<td>1 (3.2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>MRI of spine</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spinal cord hemangioblastoma</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>1.0</td>
</tr>
<tr>
<td>Vertebral body hemangioma (no. (%))</td>
<td>18 (54.5)</td>
<td>7 (20.6)</td>
<td>0.006</td>
</tr>
<tr>
<td>Number of vertebral hemangiomas per patient (median (range))</td>
<td>1 (0 - 7)</td>
<td>0 (0 - 3)</td>
<td>0.002</td>
</tr>
<tr>
<td><strong>CT of abdomen</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Liver</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hemangioma</td>
<td>4 (12.1)</td>
<td>1 (2.9)</td>
<td>0.2</td>
</tr>
<tr>
<td>Cyst</td>
<td>7 (21.2)</td>
<td>2 (5.9)</td>
<td>0.079</td>
</tr>
<tr>
<td>Portal vein thrombosis</td>
<td>2 (6.5)</td>
<td>0 (0)</td>
<td>0.2</td>
</tr>
<tr>
<td>Spleen</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hemangioma</td>
<td>1 (3.2)</td>
<td>0 (0)</td>
<td>0.5</td>
</tr>
<tr>
<td>Splenic vein thrombosis</td>
<td>2 (6.5)</td>
<td>0 (0)</td>
<td>0.2</td>
</tr>
<tr>
<td>Kidneys</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Renal carcinoma</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>1.0</td>
</tr>
<tr>
<td>Hemangioma/angiomyolipoma</td>
<td>1 (3.2)</td>
<td>0 (0)</td>
<td>0.5</td>
</tr>
<tr>
<td>Cyst</td>
<td>11 (33.3)</td>
<td>12 (35.3)</td>
<td>1.0</td>
</tr>
<tr>
<td>Adrenal glands</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pheochromocytoma</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>1.0</td>
</tr>
<tr>
<td>Pancreas</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cyst</td>
<td>1 (3.2)</td>
<td>0 (0)</td>
<td>0.8</td>
</tr>
</tbody>
</table>
Figure 1a.
Figure 1b

Estimated proportion surviving

Chuvash Polycythemia (N=94)

Community Controls (N=94)

P = 0.17

Age (years)
Figure 1c

Estimated Proportion Surviving vs. Age (years)

Chuvash polycythemia (N = 76)

Community controls (N = 76)

P < 0.0005
Figure 2a

![Graph showing serum VEGF levels by genotype.](image-url)

- **Serum VEGF (pg/ml)**
  - unaffected: 63
  - heterozygote: 79
  - homozygote: 126

- **P < 0.0005**

**VHL598C>T Genotype**

- unaffected
- heterozygote
- homozygote
Figure 2b

![Graph showing serum nitrite/nitrate levels for different genotypes.](image)

- **Genotype:** VHL598C>T
- **Legend:**
  - **unaffected**
  - **heterozygote**
  - **homozygote**

**Statistical Information:**
- **P > 0.2**
Figure 2c

![Graph showing serum total PAI-1 levels for different genotypes.](image)

- Serum Total PAI-1 (ng/mL)

- VHL598C>T Genotype:
  - Unaffected
  - Heterozygote
  - Homozygote

- P < 0.0005
Figure 2d

![Graph showing the serum EPO levels for different genotypes of VHL598C>T.](image)

- **Unaffected**
- **Heterozygote**
- **Homozygote**

**P < 0.0005**
Figure 2e

![Graph showing the comparison of VHL598C>T Genotype with serum transferrin receptor levels.](image)

- **P < 0.0005**
- VHL598C>T Genotype:
  - Unaffected
  - Heterozygote
  - Homozygote

**Serum Transferrin Receptor (mg/L):**
- 0.6
- 0.7
- 0.8
- 0.9
- 1.0
- 1.1
- 1.2
- 16
- 10
- 6
- 4
- 2
Figure 3a

[Graph showing the relationship between Hemoglobin (g/dL) and Erythropoietin (IU/L) for homozygote (closed circles) and unaffected (open circles) with the VHL598C>T variant.]
Figure 3b

![Graph showing serum transferrin receptor levels in relation to serum ferritin levels for Chuvash polycythemia patients with VHL598C>T mutation.](image)

- **VHL598C>T**
  - Unaffected: 
  - Homozygote: 

The graph illustrates the relationship between serum ferritin (µg/L) and serum transferrin receptor (mg/L) for individuals with and without the VHL598C>T mutation.
Congenital disorder of oxygen-sensing: association of the homozygous VHL mutation with thrombosis and vascular abnormalities but not tumors

Victor R Gordeuk, Adelina I Sergueeva, Galina Y Miasnikova, Daniel Okhotin, Yaroslav Voloshin, Peter L Choyke, John A Butman, Katerina Jedlickova, Josef T Prchal and Lydia A Polyakova