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Regulatory Function of the Anticoagulant Protein S in Patients with Chuvash Polycythemia

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Introduction

Background: Chuvash polycythemia is a hematological disorder that is present worldwide but endemic to the Chuvash population, a Turkish ethnic group located in Russia. The disorder is caused by a homozygous germline mutation (R200W) in the von Hippel Lindau gene. This mutation impairs binding of pVHL to hypoxia-inducible factor 1-alpha (HIF-1α); lack of this interaction prevents degradation of HIF-1α. The resultant upregulation of HIF-1α, even in a normal oxygen state, increases the activity of erythropoietin, thereby causing polycythemia **(Figure 1).** Affected individuals experience increased rates of arterial and venous thrombosis unrelated to the increased concentration of hemoglobin.

Aims: To determine whether upregulation of HIF-1α in patients with Chuvash polycythemia causes a decreased level of the antithrombotic agent protein S. A decreased level of protein S may explain the increased risks of arterial and venous thromboembolic events in this population.

Methods: Enzyme-linked immunosorbent assay (ELISA) will be performed to measure total and free protein S concentration in Chuvash and control plasma. Immunoblotting will be performed to confirm the ELISA measurements. Additional assays will be performed if Chuvash plasma is found to have a decreased protein S concentration.

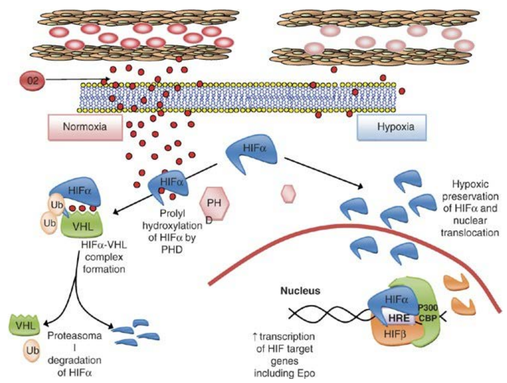

Figure 1 : Intracellular oxygen sensing and erythropoietin production. The cellular response to hypoxia is controlled by a family of transcription factors, known as hypoxia-inducible factors (HIFs). In the presence of normoxia, HIFα is rapidly destroyed by a collaborative effect of oxygen, prolyl hydroxylase domain (PHD)-containing enzymes and the von Hippel-Lindau tumor suppressor protein (VHL). Hydroxylated HIFα can bind to VHL, and the HIFα–VHL complex facilitates ubiquitin-mediated proteasomal degradation of HIFα. Under conditions of tissue hypoxia, the proteasomal degradation of HIFα is slowed, resulting in its cytoplasmic accumulation and subsequent translocation to the nucleus, where it dimerizes with HIFβ and enhances the transcription of the erythropoietin gene (Patnaik, M., Tefferi, A. The complete evaluation of erythrocytosis: congenital and acquired. *Leukemia* **23,** 834–844 (2009))

Quantitative Value for Total Protein S

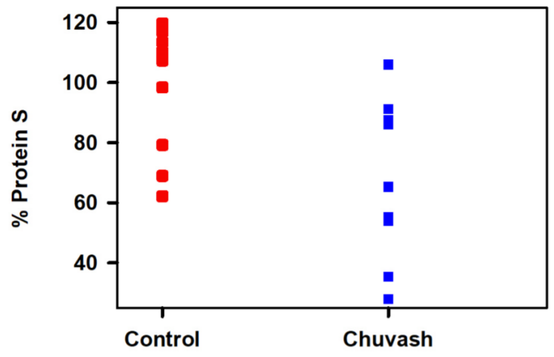

Figure 2 : ELISA was performed on control and Chuvash patient plasma using Diapharma's® REAADS Protein S Antigen Kit. The provided scatter plot shows the total amount of protein S as a percent of normal, with 100% being normal. The mean values for the control and Chuvash samples tested were 94.6% and 79.6%, respectively.

Total Protein S Immunoblotting

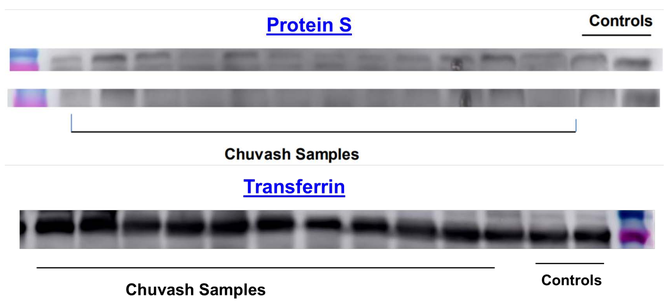

Figure 3 : Immunoblotting was performed to validate the ELISA results previously shown for the quantity of total protein S. We found band densities in Chuvash samples are *fainter* than control samples. Further, desensitization of the bands using Image J software confirmed the intensities of the controls are higher than the Chuvash samples. Thus, our immunoblot data and ELISA data suggest that there is downregulation of total protein S in Chuvash patients.

Statistical Significance

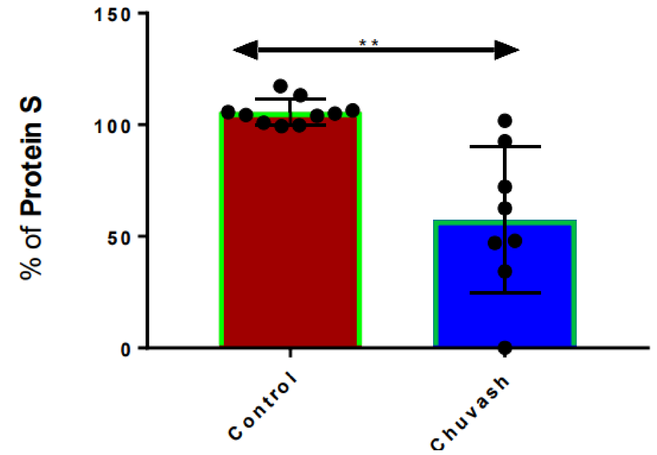

Figure 4 : Student T-test (unpaired) was performed, comparing the total protein S reported as percent of normal. We found a statistically significant difference (p value = 0.004) between the total protein S in Chuvash and control patient plasma.

Conclusions

Previous research has shown that the increased rates of arterial and venous thrombosis seen in patients with Chuvash polycythemia is independent from the increased concentrations of hemoglobin seen in this population. Our study explored a different potential cause for the increased rates of thrombosis by measuring levels of the antithrombotic protein, protein S. We observed a statistically significant decrease in the total protein S levels in Chuvash polycythemia plasma when compared to control plasma. This decrease was supported by immunoblotting for total protein S in both sets of plasma. Other studies performed by our lab have shown that upregulation of HIF-1α, as seen in Chuvash polycythemia, can decrease levels of total protein S. Decreased levels of protein S may provide a mechanism by which Chuvash patients suffer from increased rates of thrombosis.

Our future research plans are to obtain additional Chuvash plasma and measure both total and free protein S levels to expand our sample size. The samples tested in this project showed a wide range of total protein S values; by expanding our sample size we hope to better quantify the average decrease in protein S. Additionally, we will perform thrombin generation assays both with and without exogenous protein S and compare rates of thrombin generation. Long-term, we hope to develop a protein S analogue that can be administered to Chuvash patients to help prevent thrombosis in this vulnerable population.

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