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"Novel Variants Related to Protein S and Folate Deficiency in a Female Patient"

Protein S (PS) is a multifunctional protein that exists in a free and bound form in plasma. The free form of PS acts as an anticoagulant in plasma. Traditionally, PS's role in coagulation is described as a cofactor for Activated Protein C (APC) or Tissue Factor Pathway Inhibitor (TFPI). Both pathways result in decreased thrombin generation and ultimately reduced fibrin formation. Recently, it has been discovered that PS directly inhibits Factor IXa (FIXa), and thereby directly reduces thrombin generation. PS deficiency is a life-threatening condition that can result in an increased risk for developing disseminated intravascular coagulation or venous thromboembolism in patients. Additionally, folic acid is part of the vitamin B family and acts as a coenzyme in cellular reactions related to purine, pyrimidine, and methionine synthesis. Folate deficiency, leading to methionine deficiency, is related to increased levels of homocysteine, which has been shown to be a cardiovascular risk factor. We studied a 46-year-old-female with Proteins S and folate deficiency, whose mother has a history of multiple miscarriages and thrombophilia.

The aim of this study is to evaluate the genetic and proteomic data related to Protein S deficiency in the female patient. The patient was assessed for PS deficiency via quantitative assays. However, no genetic testing was ordered by the physician at that time. Platelet Poor Plasma (PPP) was isolated from the patient's citrated blood samples and stored at -80°C until the time of use. Proteomic analysis was conducted via aPTT, thrombin generation assays (TGA), immunoblotting, and enzyme linked immunosorbent assays (ELISA). Genetic analysis was conducted by isolating DNA from whole blood. Primers were designed for the human *PROS1* gene on chromosome 3. The whole gene was sequenced using Myseq system (Illumina) in 2 control samples and the patient's sample.

Quantitative tests (immunoblotting and ELISA) displayed decreased levels of total and free PS respectively. Immunoblotting revealed an 18% decrease in total PS. ELISA revealed a 46% decrease in free PS. Functional tests (aPTT and TGA) displayed decreased clotting time and increased total thrombin generation, indicating the patient has reduced levels of PS. Genetic analysis revealed 5 variations in the patient's *PROS1* gene. One of the variations (*rs6123*) has been observed to protect individuals from deep venous thrombosis, which may explain why the patient does not show symptoms of PS deficiency. The other variation, rs8178610, explains the folate deficiency in the patient since this variation is related to gene expression of *DHFRL1* in blood. *DHFRL1* is one of the enzymes capable of the reduction of dihydrofolate to tetrahydrofolate. This reaction is necessary to ensure a continuous supply of biologically active folate, reducing the risk of thrombosis. The other three variations are new intron indels which can affect *PROS1* regulation and splicing. One of them promotes the activation of a cryptic acceptor site, altering the splicing process in Protein S. Future research warrants examining the patient's family history and assess family members' genetic variations in the *PROS1* gene.