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

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

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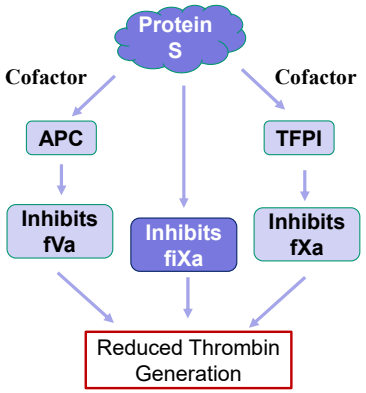
Introduction

- Protein S (PS) deficiency is a **major contributor** to acquired hypercoagulability.
- Acquired hypercoagulability causes myocardial infarction, stroke, and deep vein thrombosis in **millions of individuals**.
- Despite its importance, PS is the **least understood** anticoagulant.
- **Folate deficiency**, leading to methionine deficiency, is related to increased levels of homocysteine.
- Increased levels of homocysteine are a **cardiovascular risk factor**.



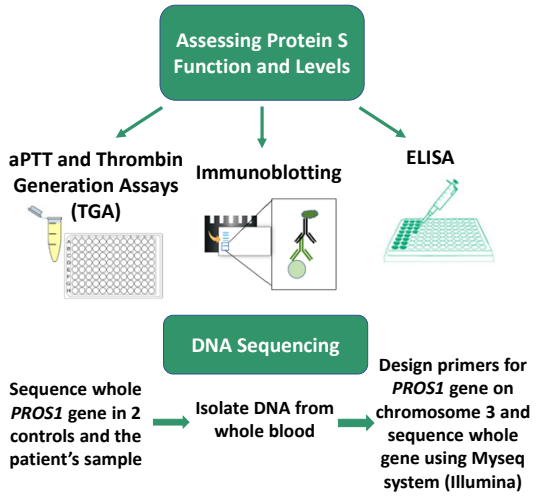
Aims

The aim of this study is to evaluate the genetic and proteomic data related to Protein S Deficiency in the female patient.

Patient History

- The patient is a 46-year-old female.
- Her mother has a history of multiple miscarriages and thrombophilia.

Methods



aPTT and TGA Analysis

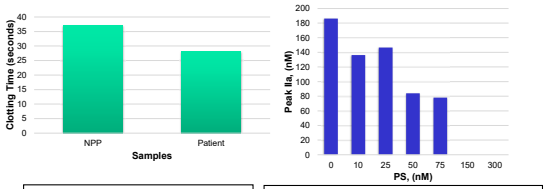


Figure 1: aPPT Clotting Time in Normal Pooled Plasma (NPP) and the patient's plasma.

Figure 2: TGA, measuring Peak IIa generation. Increasing concentrations of PS were added to the patient's plasma.

Immunoblotting Analysis

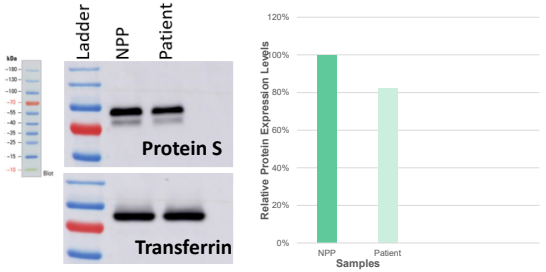


Figure 3: Immunoblot analysis of total Protein S levels in NPP and the patient's sample. Transferrin was stained for as a loading control using Sheep Anti-PS Antibody/ Anti- Sheep IgG.

ELISA Analysis

Samples	% PS
NPP	100%
Patient	57.96%

Figure 4: Amount of free Protein S in NPP and the patient's sample

DNA Sequencing Analysis

Novel Variations in *PROS1* Gene	Effect on mRNA
c.1061+62_1061+63ins	May cause skipping of exon 10
c.945+93del	May create new cryptic acceptor splice site
c.443-159del	May produce two new cryptic acceptor splice sites

Known Variations in *PROS1* Gene	Effect on mRNA
rs9681204	3' UTR variation in patient and control
rs1401681102	SNP in both patient and control
rs6123	Synonymous variation that does not affect protein structure
rs8178610	*SNP in intron related to DHFRL1- significant because may help explain the patient's folate deficiency and anemia*

Discussion

- Functional tests (aPTT and TGA) displayed **decreased clotting time** and **increased total thrombin generation.** The addition of PS lead to a decrease in Peak IIa generation, indicating the patient has reduced levels of PS. Of note, addition of 75 nM PS lead to a **41.9% decrease in Peak IIa.**
- 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 **42% decrease** in free PS.
- DNA sequencing revealed **7 variations in the *PROS1*** gene.

Conclusion

Future research warrants examining the three novel variations and conducting proteomic analyses on the altered proteins in the patient.

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