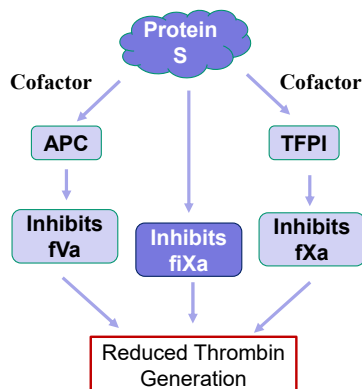


## Introduction

- 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**.



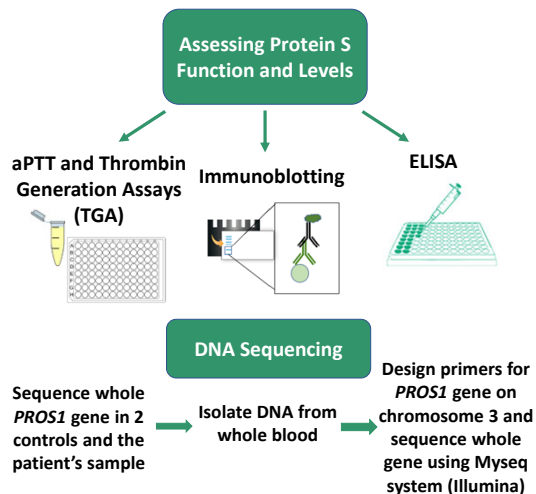
## Aims

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

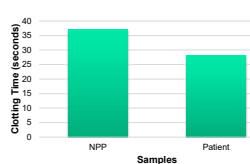


Figure 1: aPTT Clotting Time in Normal Pooled Plasma (NPP) and the patient's plasma.

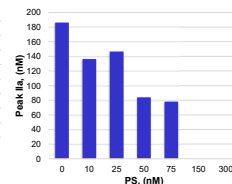


Figure 2: TGA, measuring Peak IIa generation. Increasing concentrations of PS were added to the patient's plasma.

## Immunoblotting Analysis

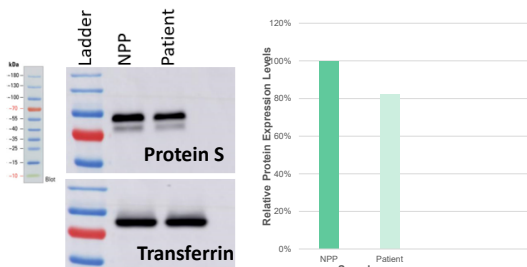


Figure 3: Immunoblot analysis of total Protein S levels in NPP and the patient's sample. Transferrin was stained for as a loading control using Sheep Anti-PS Antibody/ Anti-Sheep IgG.

## 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.