Functional characterization of a SNP (F51S) found in human alpha 1-antitrypsin

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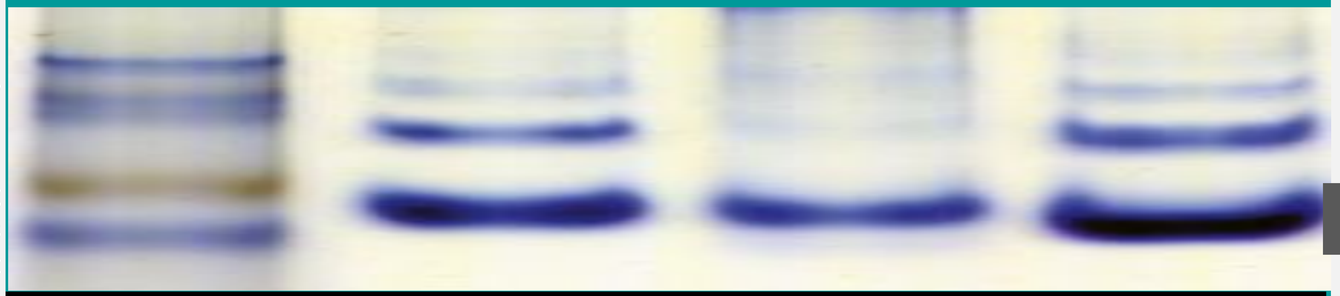
Background

- Alpha 1-antitrypsin (A1AT) is encoded by the SERPINA1 gene and is the most prominent serine protease inhibitor in plasma.
- A1AT deficiency is associated with lung and liver disorder including pulmonary bronchiectasis, emphysema, and liver cirrhosis. Genetic variations including single nucleotide polymorphisms (SNPs) of SERPINA1 are responsible for A1AT deficiency. However, the SNP characteristics are not well understood.
- The aim of the current study was to characterize a rare SNP (F51S) of SERPINA1 that introduces an additional N-glycosylation site in the N-terminal region of A1AT.

Methods

- Selection of SNPs introducing an additional N-glycosylation and site-di1. rected mutagenesis
- Protein expression and purification, SDS-PAGE, and western blot. Vectors were transfected in CHO-K1 cells, and secreted A1AT proteins were purified. A1AT protein purity was determined with Coomassie Brilliant Blue after gel electrophoresis.
- Enzymatic deglycosylation and trypsin inhibitory assay
- Protein thermal shift analysis. The melting temperature, at which 50% of the protein is unfolded, was determined with thermal shift analysis using a real-time PCR instrument.
- Acrylamide-induced quenching of Trp fluorescence. Conformational flexibility was determined by acrylamide-induced quenching of Trp and Tyr fluorescence.

The mutation F51S in the in the human alpha 1-antitrypsin gene changes its conformation and thermal stability, which may be related to its harmful effects for human health.



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Results

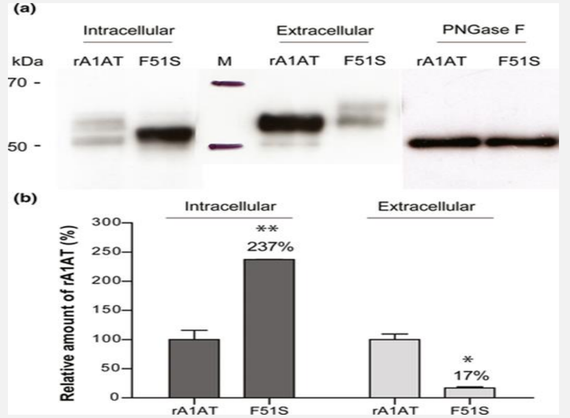

Figure 1. Expression of WT and F51S. (*p < 0.05, **p < 0.01).

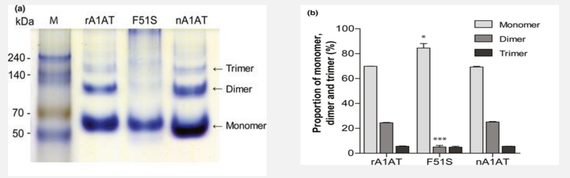

Figure 2. The polymerization level of A1AT variants. (*p < 0.05, **p < 0.01).

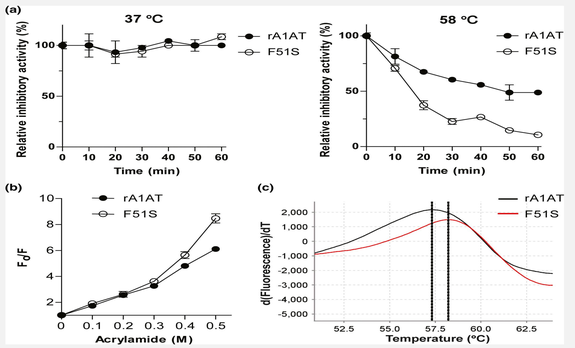

Figure 3. The thermal stability of A1AT at 37°C and 58°C.

Conclusion

➤The F51S mutation decreases A1AT secretion in CHO-K1 cells and the thermal stability of A1AT, suggesting that the F51S variant may lead to A1AT deficiency in humans.

➤The approach utilized in this study may facilitate the detection of high-risk rare SNPs.

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Results

F51S expression in CHO-K1 cells

- The F51S variant was identified as a potential N-glycosylation SERPINA1 SNP. The F51S was expressed in CHO-K1 cells; it accumulated in cells and was intracellularly 98% un-glycosylated. Six times less F51S A1AT than wild type (WT) A1AT was secreted, and it was glycosylated (Figure 1).

The inhibitory activity of the F51S variant

- The inhibitory activity of F51S and WT variants was similar.

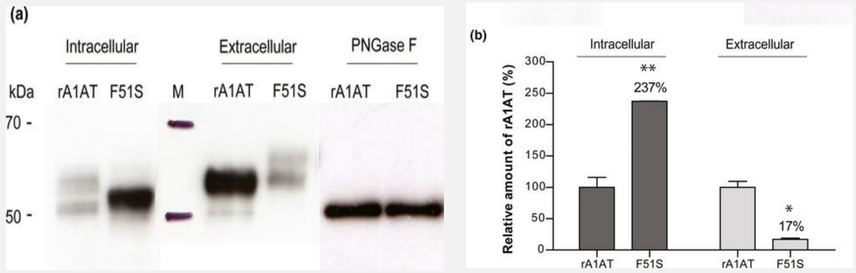

Figure 1: Expression of WT and F51S. (a) Western blot data. (b) Quantified A1AT protein levels. (*p < 0.05, **p < 0.01).

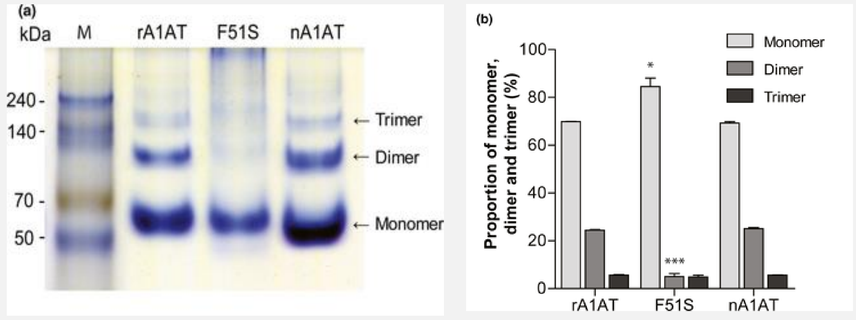

Figure 2. The polymerization level of A1AT variants. (a) Acrylamide gel separated monomers, dimers, and trimers of rA1AT, F51S, and nA1AT. (b) Proportion of monomers and polymers (*p < 0.05, **p < 0.01).

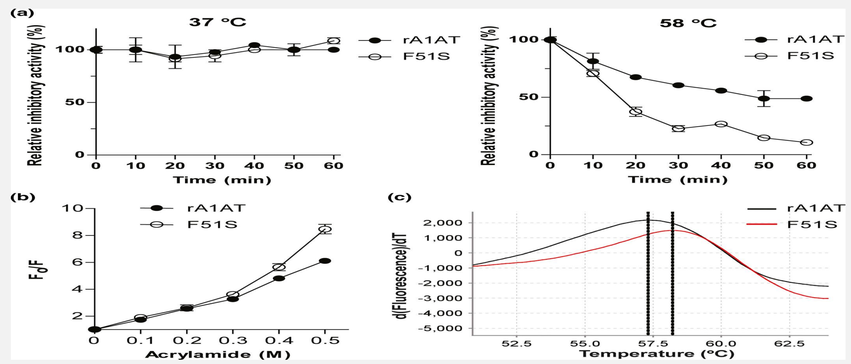

Figure 3. There was a decrease in thermal stability of F51S at 58°C in comparison to WT A1AT, which may be due to its more flexible conformation. F51S conformation was slightly strengthened toward preventing A1AT polymerization, and it reduced the thermal stability of the protein.

Conclusion

➢The F51S mutation decreases A1AT secretion in CHO-K1 cells and the thermal stability of A1AT, suggesting that the F51S variant may lead to A1AT deficiency in humans.

➢The approach utilized in this study may facilitate the detection of high-risk rare SNPs.