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Characterization of a dysfunctional antithrombin variant
"Antithrombin III Aalborg"

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Introduction. An AT variant denoted AT-III Aalborg has been identified in patients suffering form venous thromboembolic disease. The variant is a type II AT deficiency with normal antigen and decreased activity levels, but only when measured with an anti-IIa method whereas anti-Xa activity is normal. The aim of this study was to elucidate and characterize AT-III Aalborg through a biophysical and proteomic approach.

Method. To understand the cause of the dysfunction of this AT-variant several biophysical experiments and mass spectroscopic analyses were performed:

- Identification of the disease-related modification by mass spectrometry (MS) and DNA-diagnostics
- Estimation of the rate constants for inhibition of thrombin and factor Xa
- Estimation of the heparin binding affinity with Fluorescence spectroscopy
- Investigation of the conformational stability of AT-III Aalborg by circular dichroism
- Detection of Polymers by Dynamic light scattering.
- Conformation and structural analysis by electrophoresis.

Results. DNA-analysis and MS showed a serine to leucine conversion at position 394, i.e. in the reactive loop. MS did not indicate any other changes. The inhibition rate constant of AT-III Aalborg for IIa was $1/5^{th}$ of the normal whereas for Xa it was half of the normal. Heparin binding affinity was normal. Biophysical investigations did not indicate a changed conformational stability

Conclusion. AT-III Aalborg has a change in the reactive loop which lowers the inhibition rate of IIa much more than expected. If the mutated AT had no effect at all, a rate constant of half the normal would be expected. This indicates that the mutated AT not only does not inactivate thrombin, but it inhibits the effect of the wt AT, further indicating that a type II AT deficiency may be more severe than a type I deficiency which may explain the severe phenotype of AT-III Aalborg.