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Supplemental Data

The Infective Polymerization of Conformationally Unstable Antithrombin Mutants May Play a Role in the Clinical Severity of Antithrombin Deficiency

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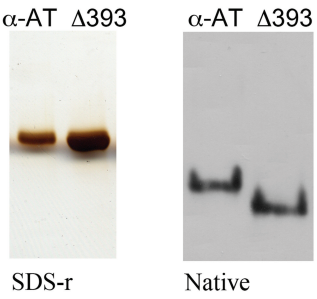
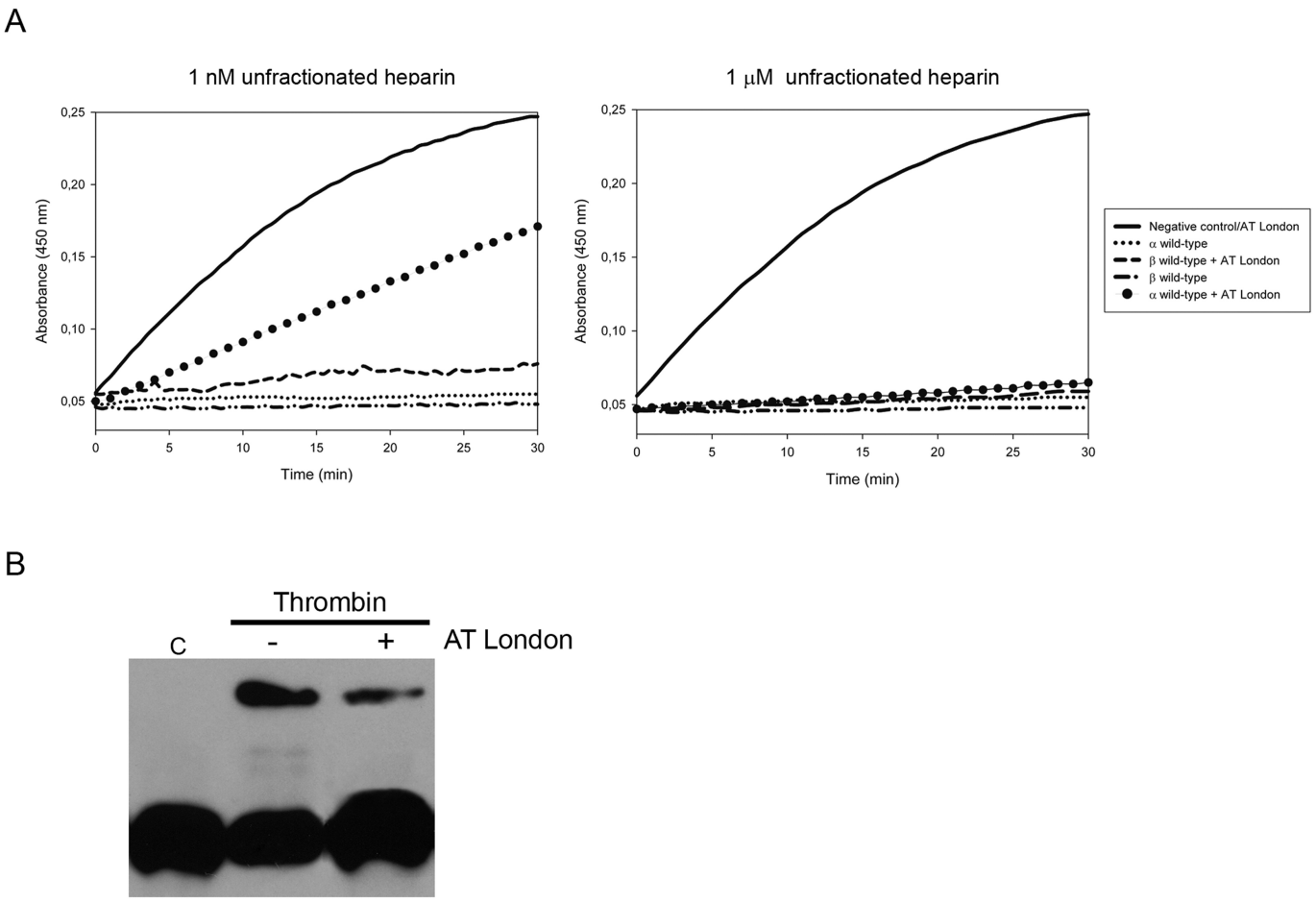
Supplementary Table S1. Plasma anti-FXa activity, antigen levels of antithrombin and clinical features of patients with antithrombin deficiency included in this study.

Mutation	Carriers	Antigen* %	Anti-FXa* %	Family history of thrombosis	Thrombosis	Age of first event
Δ393	4	86	52	Yes	AMI/AMI/PE	39/39/30
Δ393	4	98	58	No	DVT	17
R393H	4	80	50	Yes	CVD/DVT	61/0
R393C	2	89	51	Yes	DVT/DVT	57/27
M338T	6	71	61	Yes	PE/DVT/DVT/DVT	17/20/25/36
F239S	3	48	39	Yes	DVT/DVT/DVT	19/19/27

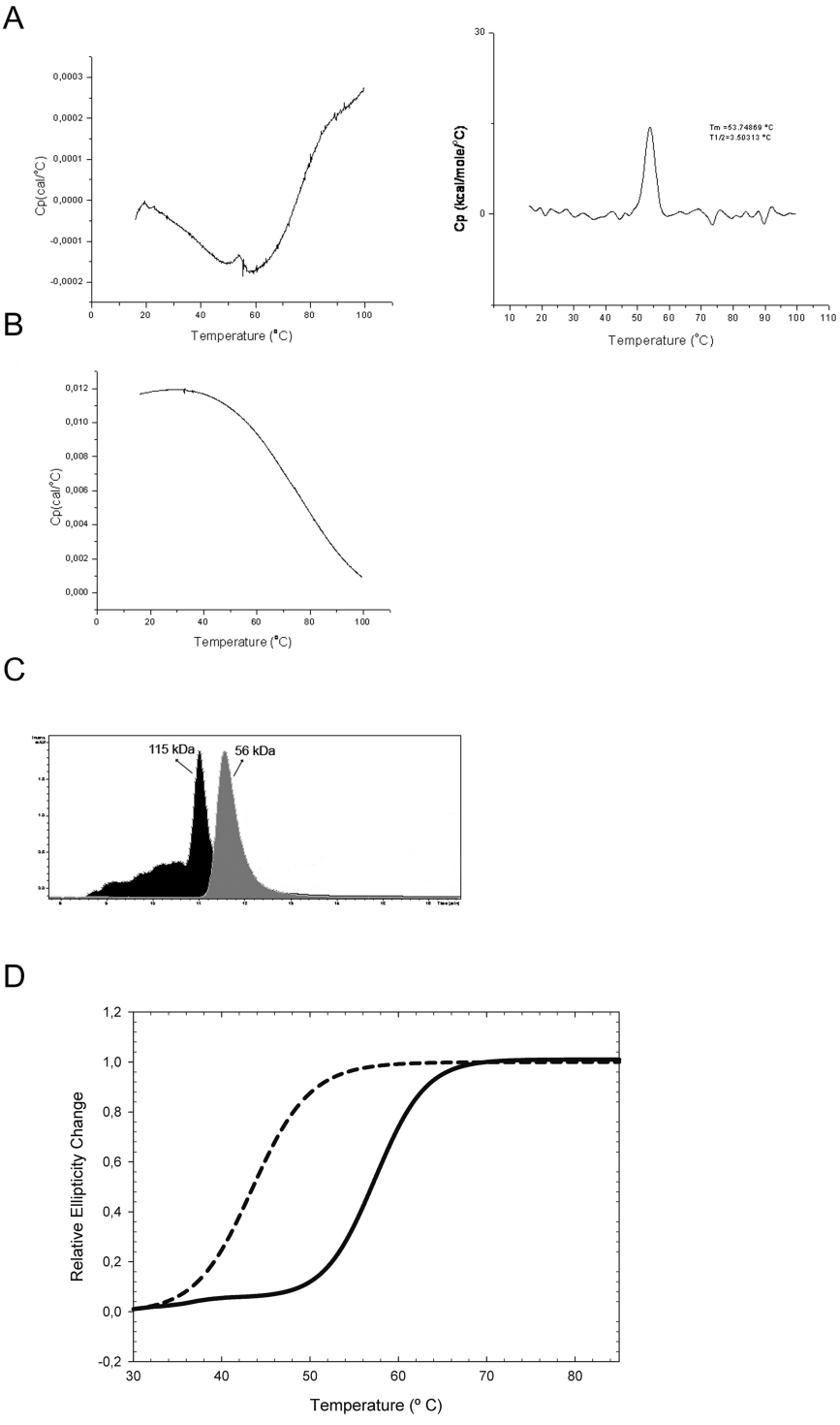
AMI: Acute myocardial infarction; PE: Pulmonary Embolism; CVD: Ischemic Cerebrovascular Disease; DVT: Deep Venous Thrombosis
* Mean of the values observed in carriers of the family.



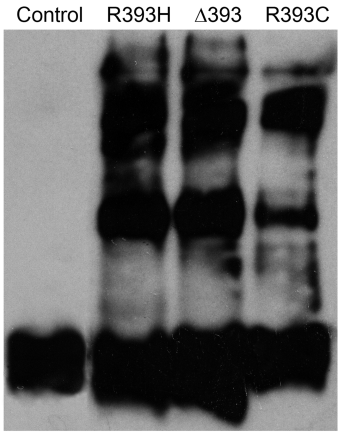
Supplementary Figure S1. Electrophoretic mobility of antithrombin London. A) SDS under reducing conditions and silver staining of purified α-antithrombin and antithrombin London (Δ393). B) Non-denaturing electrophoresis and identification by western blot of purified α-antithrombin and antithrombin London (Δ393). Lanes were loaded with 150 ng protein.



Supplementary Figure S2. Loss of inhibitory function and prothrombotic gain of function associated with antithrombin Δ393. A) Time course inhibition of thrombin by antithrombin evaluated by a chromogenic method in a continuous method in the presence of two different amounts of unfractionated heparin. Antithrombin concentration was 10-fold higher than thrombin to asses pseudo-first order conditions. B) Thrombin-antithrombin complexes detected by SDS-PAGE and western blot. The formation of thrombin-antithrombin complex was evaluated by incubation of 150 ng α-antithrombin with 0.25 U thrombin and incubated at 37 °C.



Supplementary Figure S3. Conformational instability of ΔR393 antithrombin (Antithrombin London). A) Plot obtained by differential scanning calorimetry (DSC) of wild type antithrombin (left) and melting temperature calculated after data fitting (right). B) Plot obtained by DSC of antithrombin London where no thermal transition was detected. C) Gel filtration chromatography of London (black) and wild type (grey) antithrombins previous to DSC measurements. D) Thermal stability of antithrombin London (dashed line) and wild type (continuous line) measured by changes in circular dichroism at 222 nm.



Supplementary Figure S4. Immunostaining of the same membrane shown in figure 2C: 2.5 μM ^{125}I-radiolabeled wild type antithrombin added to 1μL control plasma and 1μL plasma of carriers of P1 mutants heated at 42°C up to 3 d. Samples were run in native gels.