

## **MATERIAL SUPPLEMENTARY**

### **Methods**

#### 1. Complement parameters

Blood samples were collected using sodium citrate tubes and centrifuged (20 min, 2000 g, 22°C). The plasma samples were immediately frozen and stored at -80°C until tested. C1 inhibitor (C1-INH) activity was measured using a colorimetric assay (Technochrome C1-INH, Technoclone GmbH, Wien, Austria). Normal values of activity of C1-INH are greater than 0.7 Unit C1-INH/ml (>70%). C1-INH and C4 antigen levels were measured by means of radial immunodiffusion (RID) (NOR-Partigen, Siemens Healthcare Diagnostics, Munich, Germany).

#### 2. Anti-C1-INH antibodies detection

Autoantibodies to C1-INH were detected using a home-made enzyme-linked immunosorbent assay (ELISA), as described previously [1]. Briefly, an ELISA plate was coated using a solution of plasma derived C1-INH in phosphate buffered saline (PBS) buffer. After washing, each serum sample was diluted 1:10 in PBS-Tween20 0.05%, added to the wells and incubated for 1 hour. Isotype determination of antibodies in samples was performed by testing the binding of anti-human IgA, IgM, and IgG antibodies to anti-C1-INH antibodies linked to C1-INH fixed to the plate. The assay was also performed coating the plate with recombinant C1-INH diluted in PBS buffer.

### 3. Anti-C1q antibodies

Anti-C1q autoantibody levels were measured using a home-made ELISA. Briefly, microtiter plates (MaxiSorp®, Nunc, Denmark) were coated with 25 µg/ml of human C1q (Calbiochem, Merck, Germany), in sodium carbonate buffer for 1 hour at 37 °C and overnight at 4°C. Microtiter wells were incubated with patients' sera diluted in phosphate buffer. The samples were incubated for 1 hour at 37°C. After washing, horseradish peroxidase (HRP)-conjugated goat anti-human IgG/IgA (Sigma-Aldrich/Merck, Germany) were applied in 1:1000 dilution and incubated for 1 hour at 37°C. After washing three times, the colour was developed with o-phenylenediamine (OPD). The reaction was stopped with a 10% solution of H<sub>2</sub>SO<sub>4</sub> in water and absorbance at 490 nm was measured using a plate reader.

### 4. Genotyping

Genomic DNA was isolated from peripheral blood leukocytes according to standard protocols. Mutational screening of *SERPING1*, *ANGPT1*, *PLG*, *MYOF*, *KNG1* e *F12* was performed by direct DNA sequencing as described elsewhere [2].

### 5. Quantification of functional C1-INH in plasma based on inhibition of kallikrein, factor XIIa and C1s

Quantification of functional C1-INH based on inhibition of kallikrein and factor XIIa has been described previously [3]. Briefly, ELISA plates were coated with 1 µg/ml streptavidin (S4762, Sigma-Aldrich) in carbonate coating buffer overnight at 4°C. Plates were washed three times using PBS-Tween. Subsequently, 1% bovine serum albumin (BSA) in PBS was added to block the unused sites.

The plates were incubated at 37°C for 1 hour and washed again. Samples or standards were added to

the plates along with biotinylated enzymes (25  $\mu$ l standards or samples, 25  $\mu$ l at 1  $\mu$ g/ml biotinylated factor XII, kallikrein or C1s, and 50  $\mu$ l binding buffer). Plates were mixed and incubated at 37°C for 1 hour. After washing a goat polyclonal antibody to C1-INH (C8159, Sigma-Aldrich) was added, followed by incubation at room temperature (RT) for 1 hour. The wells were washed again, an anti-goat IgG HRP (A5420, Sigma-Aldrich) antibody was added, and the plate incubated at RT for 1 hour. Finally, the reaction was developed with TMB (3,3',5,5'-Tetramethylbenzidine) peroxidase substrate (KPL, Gaithersburg, MD, USA) and stopped with 2 M H<sub>2</sub>SO<sub>4</sub>. The absorbance at 450 nm was read, and calculations were performed using the standard curve.

#### 6. Cleavage of high-molecular weight kininogen

The cleavage of high molecular weight kininogen (HK) was assessed in sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and immunoblotting analysis. The amount of cleaved (cHK) was expressed as a percentage of total HK [4].

#### 7. Purification of the IgG of the patient

IgG of the patient were purified by affinity chromatography on protein G. A homemade column with Protein G-Agarose resin (Sigma P7700) was connected to a fast protein liquid chromatography (FPLC) device and equilibrated in PBS-0.5 M NaCl. Plasma samples (300 $\mu$ l) were loaded at a flow rate of 0.5 ml/minute. After extensive washing, bound IgG were eluted with 0.1 M glycine, pH 2.7 and immediately neutralized with 1.5 M Tris pH 8.75. Purified IgG were desalted and concentrated using Amicon ultra-15 50K.

### 8. Kinetic assay for neutralizing activity of purified IgG towards C1-INH

Kinetic test for plasma kallikrein and C1s with C1-INH were set up using the following conditions. For kallikrein: [PKa] = 0.025 nM; [C1-INH] = 25 nM; [PFR] = 250  $\mu$ M. For C1s: [C1s] = 10 nM; [C1-INH] = 10 nM; [RGR] = 250  $\mu$ M. Purified IgG were assayed at different concentration ranging from 5  $\mu$ M to 500  $\mu$ M. Complexes between IgG ([10  $\mu$ M to 1mM]) and C1-INH ([50nM] or [20nM]) were allowed to form for 1h at 25°C or 37°C or overnight at 37°C.

### 9. Purification of anti-C1-INH antibodies of the patient

Autoantibodies against C1-INH were purified from IgG fractions by affinity chromatography on C1-INH. C1-INH was coupled to sepharose and coupled resin was transferred into Micro Columns (Micro Bio-Spin™ Chromatography Columns Bio-Rad). Autoantibodies were allowed to bind in PBS buffer, subsequently eluted with 0.1 M glycine pH 2.7 and immediately neutralized with 1.5 M Tris pH 8.75. Amount of purified antibodies was quantified spectroscopically.

### 10. ELISA for neutralizing capacity of anti-C1 inhibitor antibodies

In order to study neutralizing capacity of anti-C1-INH antibodies, the ability for C1-INH to bind C1s, kallikrein and FXIIa was evaluated. In these assays, as described previously [5], ELISA plates (MaxiSorp®, Nunc, Denmark) were coated with 10  $\mu$ g/ml purified C1-INH in PBS. After washing, Quality Controls (QC) and test samples were added in a 1:10, 1:100, 1:1000 and 1:10000 dilution in PBS/0.1% Tween20/10 mM EDTA. These were incubated for 1 hour at RT, while shaking. Any present C1-INH antibodies in these samples were hereby allowed to bind and potentially interfere with binding of ligands as applied in the next step of the ELISA. After washing, wells were incubated

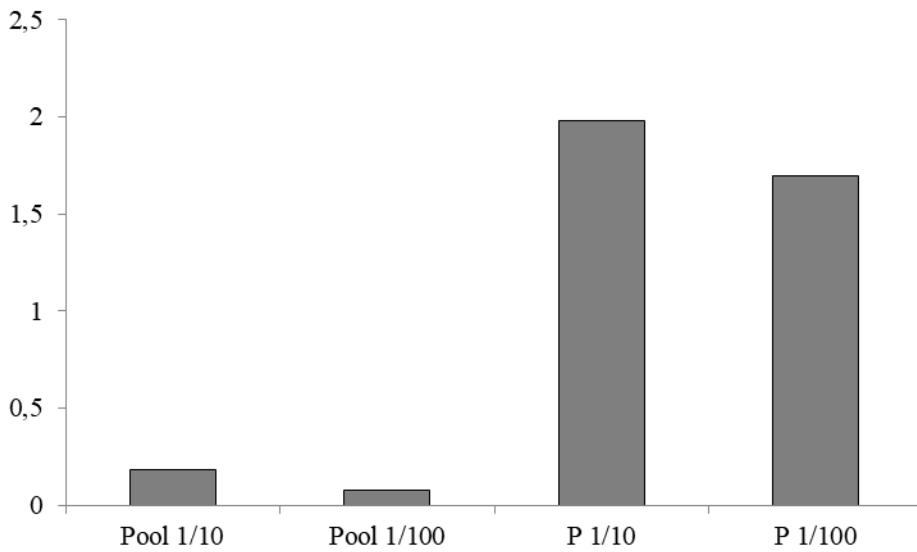
with either biotinylated C1s, kallikrein or FXIIa for 1 hour at RT, while shaking. Again after a washing step, streptavidin-HRP was allowed to bind the biotinylated ligands for 30 min at RT while shaking and after washing, TMB was added as a substrate. The reaction was stopped with 2 M H<sub>2</sub>SO<sub>4</sub> and the absorbance was assessed at 450 and 540 nm. In each test three QC samples were assessed: QC1 (6 µg/ml Rabbit polyclonal anti-C1-INH diluted 1:10 in assay buffer), QC2 (12 µg/ml Rabbit polyclonal anti-C1-INH diluted 1:10 in assay buffer), QC3 (pooled human EDTA plasma collected from 400 blood donors, diluted 1:10 in assay buffer). QC3 is used to determine the background of the assay and is used in the calculation of neutralizing capacity. The percentage neutralizing capacity was calculated as follows:

Percentage neutralizing capacity:

$$(1 - A_{\text{sample}}/A_{\text{QC3}}) \times 100$$

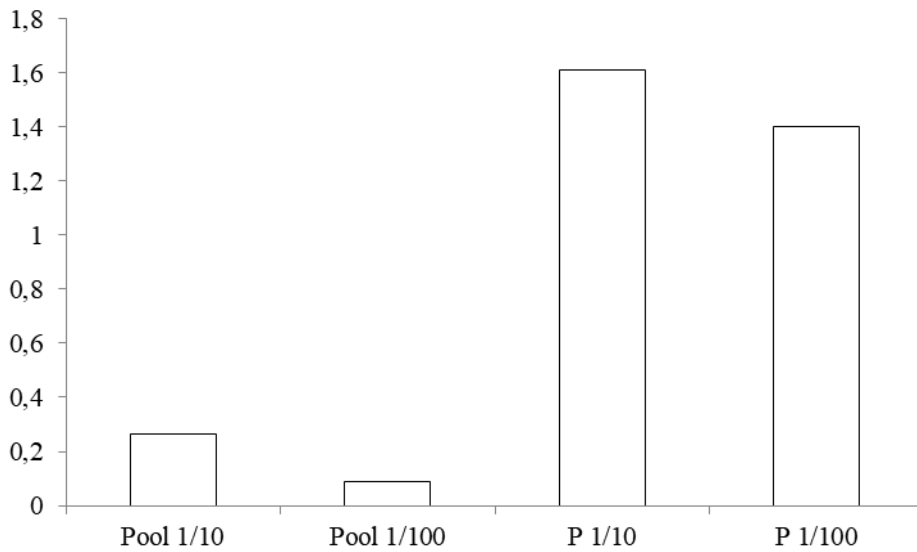
where  $A_{\text{sample}}$  = Absorbance ratio 450/540 nm of the sample at a given dilution

$A_{\text{QC3}}$  = Absorbance ratio 450/540 nm of the QC3 standard.



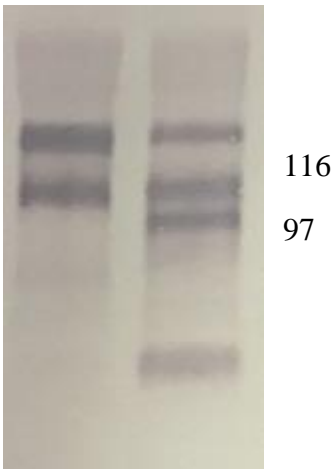
### Supplementary fig.1

Values of absorbance at 490 nm measured by means of an ELISA for the detection of autoantibodies to C1-INH (IgG). The plate was coated with a solution of plasma derived C1-INH. Pool 1/10, Pool 1/100: pool of plasma collected from 50 healthy donors, which was diluted 1/10 and 1/100 respectively. P 1/10, P 1/100: plasma of the patient diluted 1/10 and 1/100 respectively.



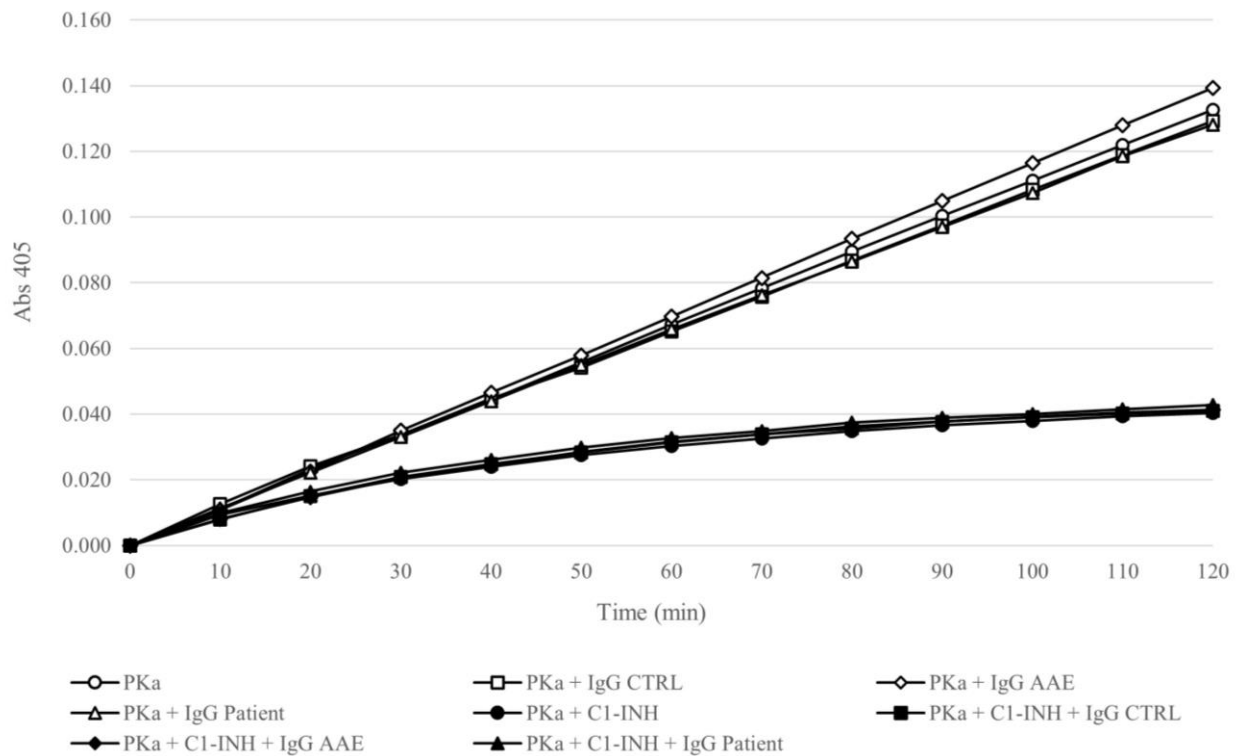
### Supplementary fig.2

Values of absorbance at 490 nm measured by means of an ELISA for the detection of autoantibodies to C1-INH (IgG). The plate was coated with a solution of recombinant C1-INH. Pool 1/10, Pool 1/100: pool of plasma collected from 50 healthy donors, which was diluted 1/10 and 1/100 respectively. P 1/10, P1/100: plasma of the patient diluted 1/10 and 1/100 respectively.

**P****Supplementary fig.3**

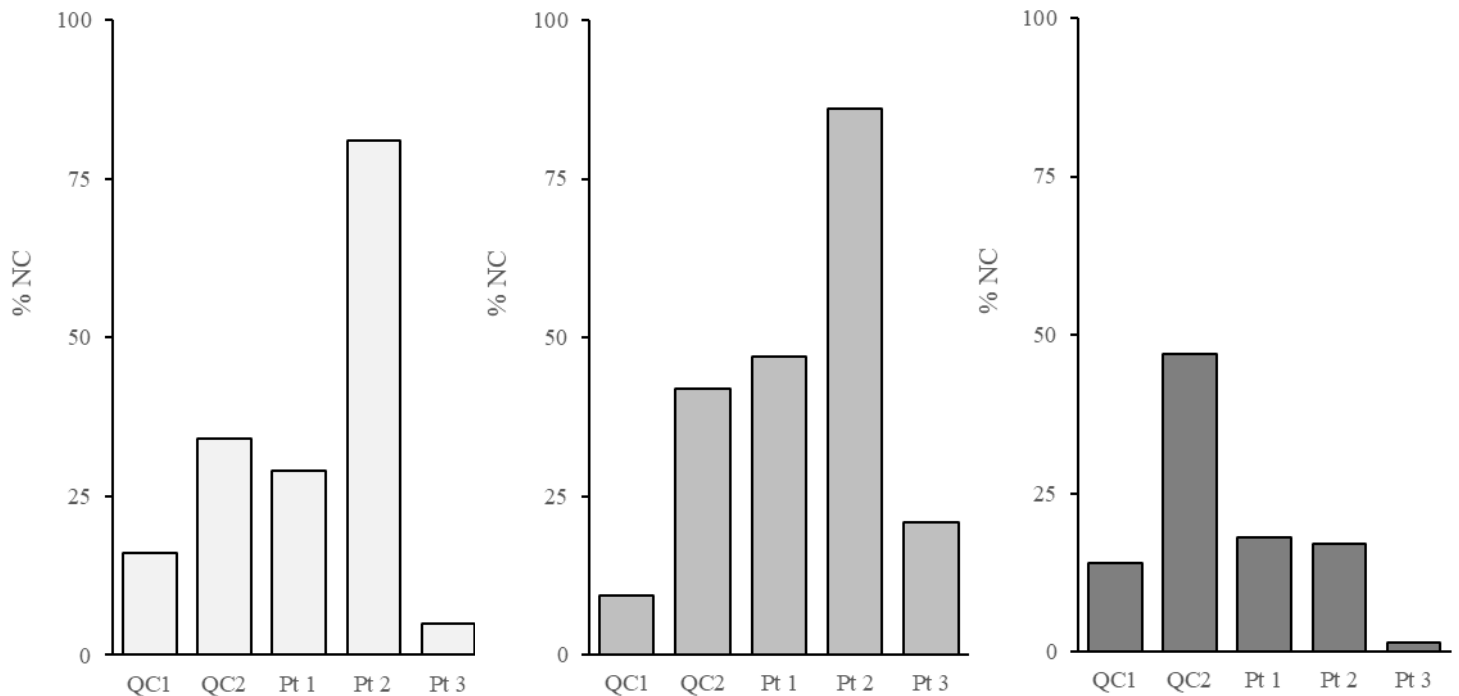
Immunoblotting of cleaved HK in plasma collected from a normal subject (N) and from the patient (P). The normal pattern is a major band with a molecular weight of 130 kDa and a second band with a molecular weight of 107 kDa; the patient showed a reduction in the band of 130 kDa and the appearance of a band with a molecular weight of 98 kDa.





#### Supplementary fig.4

Kinetic of C1-INH inhibition of kallikrein in the presence of IgG purified from patient's serum. PKa: active plasma kallikrein, IgG CTRL: IgG purified from a healthy control, IgG AAE: IgG purified from a patient with acquired AE and positive for anti-C1-INH autoantibodies; IgG Patient: IgG purified from the index patient. Top group: all open symbols. Bottom group: all closed symbols. No differences can be distinguished between the kinetic of C1-INH inhibition of kallikrein in the presence of IgG purified from patient's serum and IgG purified from a healthy control.



**Supplementary Fig 5a – c:** ELISA to evaluate the neutralizing capacity of anti-C1-INH antibodies of the patient. **a:** neutralizing capacity for C1s. **b:** neutralizing capacity for kallikrein. **c:** neutralizing capacity for FXIIa. QC1: 6  $\mu\text{g/ml}$  rabbit polyclonal anti-C1-INH, QC2: 12  $\mu\text{g/ml}$  Rabbit polyclonal anti-C1-INH, Pt 1: index patient, Pt 2: strong positive patient (patient with acquired C1-INH deficiency and anti-C1-INH antibodies), Pt 3: negative patient. %NC: percentage neutralizing capacity

**References (Supplementary Material)**

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