C3G is characterized by increased pro-factor D accumulation compared with aHUS

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Background

Factor D (FD) is the rate-limiting protease of the alternative complement pathway and circulates as an inactive precursor (pro-FD) that requires MASP-3-mediated activation. Conventional assays measure only total-FD and cannot distinguish between precursor and active enzyme.

C3 glomerulopathy (C3G) and atypical hemolytic uremic syndrome (aHUS) are alternative pathway-mediated kidney diseases with distinct pathophysiology.

We hypothesized that separating total-FD into pro-FD and active-FD would reveal disease-specific differences in FD processing. We also investigated the effect of Zaltenibart (formerly OMS906), an mAb targeting MASP-3, on *ex vivo* pro-FD conversion.

Assay development

- Monoclonal antibodies specific for total-FD, pro-FD, active-FD, and MASP-3
- Specificity confirmed by Western blot and sandwich ELISA
- Stable across five freeze-thaw cycles
- Validated for human serum and plasma

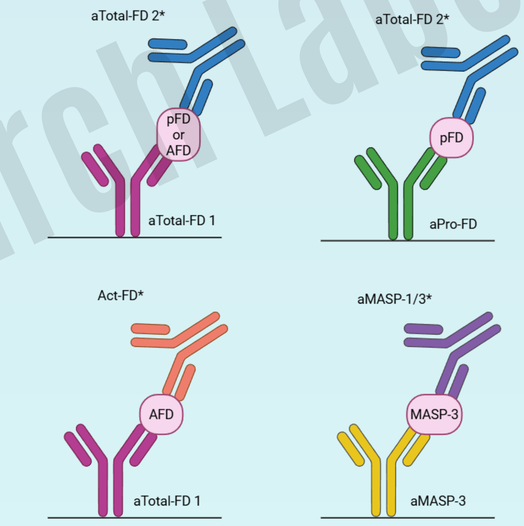

Figure 1. Novel immunoassays for quantification of total-FD, pro-FD, active-FD, and MASP-3.
Schematic illustration of the sandwich immunoassays developed in this study. Total-FD was measured using a pan-FD capture antibody recognizing both pro-FD and active-FD together with a pan-FD detection antibody. Pro-FD and active-FD were quantified using form-specific capture antibodies recognizing only the precursor or mature enzyme, respectively, combined with pan-FD detection antibodies. MASP-3 was measured using a monoclonal capture antibody and a polyclonal detection antibody.

Absolute concentrations

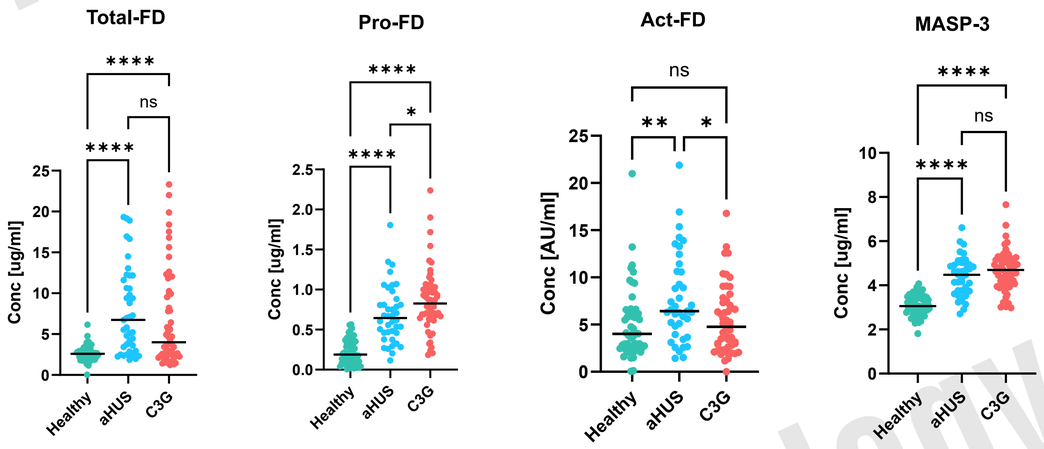

Figure 3. Absolute concentrations of total-FD, pro-FD, active-FD, and MASP-3 in C3G and aHUS.
Novel immunoassays were used to quantify serum total-FD, pro-FD, active-FD, and MASP-3 in healthy controls (n = 60), aHUS (n = 42), and C3G (n = 58). Both kidney diseases exhibited significantly increased circulating concentrations of all analytes compared with healthy controls, while pro-FD was the only marker that differed significantly between C3G and aHUS. Individual patients are shown as dots; horizontal bars represent the median. Statistical significance was determined by one-way ANOVA with Tukey's multiple comparisons test.

Total-FD, pro-FD, active-FD, and MASP-3 concentrations were significantly elevated in both C3G and aHUS compared with healthy controls. Total-FD, active-FD, and MASP-3 did not differ significantly between C3G and aHUS. Pro-FD was significantly higher in C3G than in aHUS ($p < 0.05$). These findings suggest that although both diseases exhibit increased circulating FD, C3G is characterized by greater accumulation of the inactive precursor pro-FD.

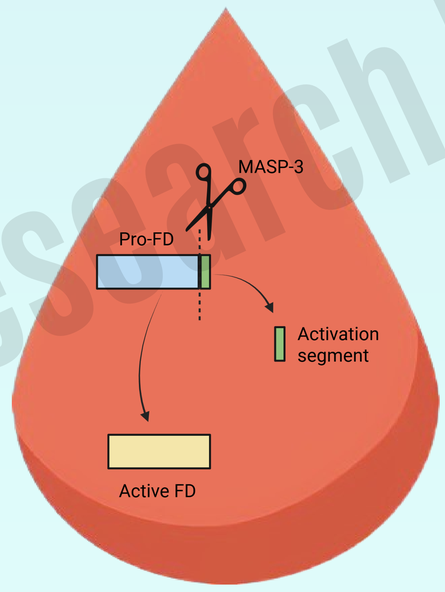

Figure 2. Extracellular activation of factor D by MASP-3.
Factor D (FD) is synthesized and secreted by adipocytes as the inactive precursor pro-factor D (pro-FD). Following secretion into the extracellular space, MASP-3 cleaves the N-terminal activation peptide to generate enzymatically active factor D (active-FD), which is capable of initiating alternative pathway amplification.

Relative concentrations

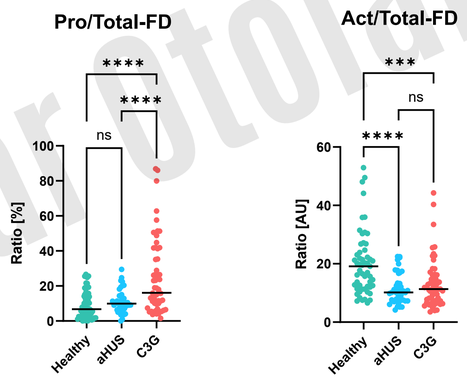

Figure 4. Disease-specific differences in FD processing in C3G and aHUS.
Ratios of circulating FD forms were calculated to assess FD processing independent of total-FD concentration. C3G demonstrated greater accumulation of inactive pro-FD relative to total-FD than aHUS, whereas both diseases exhibited a reduced proportion of active-FD compared with healthy controls. Statistical significance was determined by one-way ANOVA with Tukey's multiple comparisons test.

C3G exhibited a significantly higher pro-FD/total-FD ratio than both healthy controls ($p < 0.0001$) and aHUS ($p < 0.0001$). The active-FD/total-FD ratio was reduced in both C3G ($p < 0.001$) and aHUS ($p < 0.0001$) compared with healthy controls, with no significant difference between the disease groups.

Pro-FD conversion kinetics

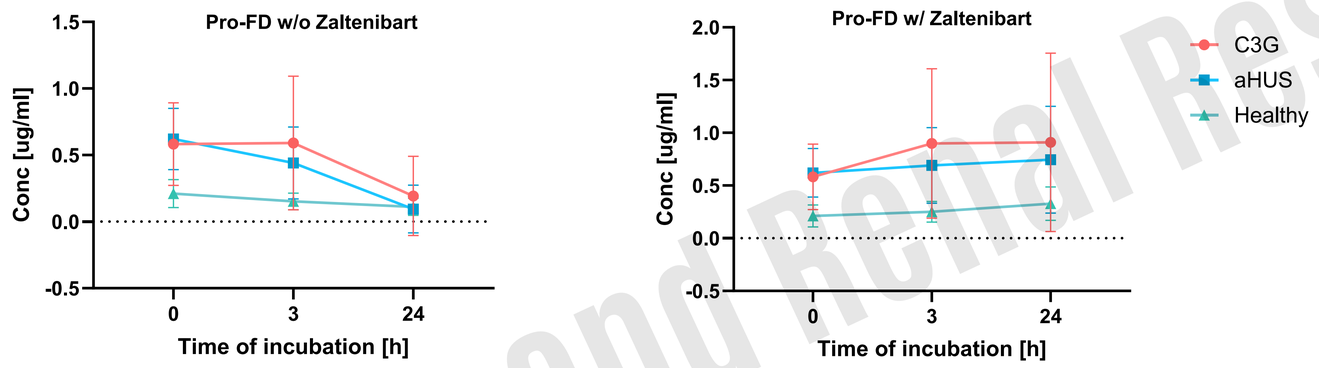

Figure 5. *Ex vivo* pro-FD conversion kinetics in healthy controls, aHUS, and C3G.
Ex vivo conversion of pro-FD was assessed by incubating serum from healthy controls, patients with aHUS, and patients with C3G at 37 °C for 0, 3, and 24 h in the absence or presence of 0.0025 µg/mL zaltenibart. Pro-FD concentrations were measured using the pro-FD-specific immunoassay at each timepoint. In the absence of inhibitor, pro-FD consumption was greatest in aHUS, intermediate in C3G, and lowest in healthy controls. Addition of zaltenibart attenuated pro-FD consumption in all three groups, consistent with inhibition of alternative pathway activity. Data are presented as mean ± SD (n = 11 per group).

Pro-FD was consumed during incubation in all groups. aHUS exhibited the greatest pro-FD consumption, whereas conversion was less pronounced in C3G. Zaltenibart markedly reduced pro-FD consumption in all groups. These findings support disease-specific differences in FD activation dynamics and complement the altered FD processing observed in vivo.

Conclusions

Both C3G and aHUS exhibited elevated circulating FD, but FD processing differed between the diseases. C3G was characterized by increased pro-FD accumulation and a higher pro-FD/total-FD ratio, suggesting relatively impaired FD maturation. *Ex vivo* kinetics supported these findings, with slower pro-FD consumption in C3G than in aHUS and inhibition of pro-FD conversion by zaltenibart. Measuring pro-FD and active-FD provides mechanistic information beyond total-FD alone.

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