

# Association of Complement Factor D with Cardiovascular Mortality and Clot Lysis in CKD

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Patients with CKD are at a higher risk of cardiovascular and thrombotic events that are linked to a procoagulatory phenotype.<sup>1</sup> In acute coronary syndrome, CKD is associated with a denser clot structure and prolonged clot lysis.<sup>2</sup> In addition, clot structure is a significant predictor of mortality in CKD.<sup>3</sup> Notably, the complement system and CKD are closely interconnected, and complement activation products, found in urine of patients with CKD, are associated with severity of glomerular disease and impairment of kidney function.<sup>4,5</sup> Despite accumulating evidence on complement activation and thrombosis, it remains unclear which complement pathway (*i.e.*, classical, lectin, or alternative) is activated in CKD. Therefore, we sought to investigate the association between complement proteins and their respective pathways in CKD, as well as explore their role in contributing to a prothrombotic coagulation phenotype.

The analysis was based on the UK Biobank, a prospective cohort of approximately 500,000 participants recruited between 2006 and 2010 in the United Kingdom. Proteomic data of complement proteins using the Olink Explore 3072 was available for 44,856 individuals. Cardiovascular events were defined using International Classification of Diseases–coded hospital admissions and mortality records, including cardiovascular death (death related to International Classification of Diseases 100–199 codes), myocardial infarction (I21–I22), and stroke (I60–I69). The median observation time was 15.7 years (interquartile range, 14.9–16.5 years). Fibrin clot properties were assessed using a validated turbidimetric assay in citrate plasma, measuring maximum absorbance to evaluate clot density and determining clot lysis time from full clot formation to 50% lysis. For validation studies, blood

samples from healthy individuals and patients with CKD undergoing hemodialysis were assessed. All participants provided informed consent and were free from infection and anti-inflammatory treatment. All experimental studies were approved by the Ethics committee of University Hospital Aachen (EK 233/19, detailed description in [Supplemental Material](#)).

Among all available complement proteins, the rate-limiting alternative pathway (AP) protein complement factor D (CFD) demonstrated the strongest correlation with eGFR (calculated by CKD-Epidemiology Collaboration) with an R of 0.36 ( $P < 0.001$ ; [Figure 1A](#)). Therefore, CFD was the focus of all subsequent analysis. Indeed, high plasma levels of CFD are commonly observed in CKD and have been demonstrated to increase AP activity by increased facilitation of the fluid phase C3 convertase formation from spontaneous hydrolyzed C3(H<sub>2</sub>O).<sup>6</sup> To assess the role of CFD on cardiovascular disease and thrombotic end points, we stratified patients into tertiles and assessed major adverse cardiovascular events (MACE), including cardiovascular death, nonfatal myocardial infarction, and nonfatal stroke ([Figure 1B](#)). After adjustment for age, sex, diabetes, hypertension, myocardial infarction, coronary artery disease, N terminal pro-B-Type natriuretic peptide, eGFR, high sensitivity C-reactive protein, body mass index, and urine albumin-creatinine ratio, CFD remained an independent predictor of MACE (hazard ratio, 1.44 [95% confidence interval, 1.18 to 1.75], [Figure 1B](#)) as well as of the MACE components, cardiovascular death and nonfatal myocardial infarction. To investigate the pathologic relation between CFD and eGFR, we stratified patients into risk groups according to CFD tertiles and CKD stages (*i.e.*, CKD G1-2 A1, CKD G1-2 A2-3, and CKD

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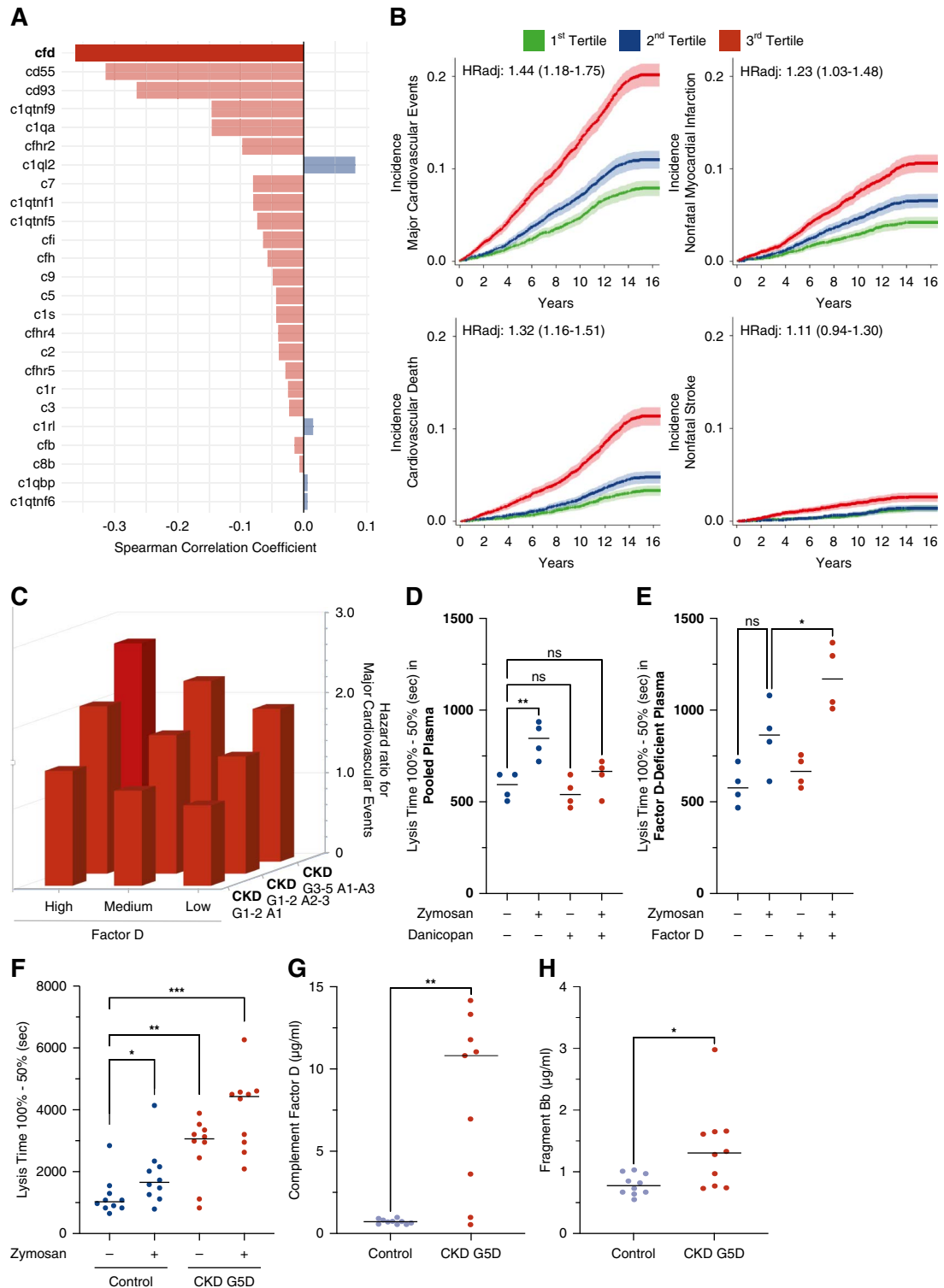
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**Figure 1. Increased levels of complement factor D predict major cardiovascular events and link alternative pathway activation to a procoagulatory phenotype with increased resistance to clot lysis.** (A) Spearman correlation plot of complement proteins assessed in the UK Biobank. OLINK 3072 release. Proteomic data available for 44,856 patients. Red bars denote positive and blue bars denote negative correlation. (B) Incidence of MACE according to CFD tertiles. Incidence truncated at last event. Upper left, MACE. Lower left, cardiovascular death. Upper right, myocardial infarction. Lower right, stroke. Cox proportional hazards models are adjusted for age, sex, diabetes, hypertension, myocardial infarction, coronary artery disease, NTproBNP, eGFR, hsCRP, BMI, and UACR. Log transformations and restricted cubic splines were used where appropriate to account for nonlinear relationships and skewed distributions. Interactions between included variables were analyzed and incorporated into the models as necessary. (C) Hazard ratios for MACE stratified by CFD tertiles and presence and absence of CKD (*i.e.*, CKD G1-2 A1 [eGFR >60 ml/min and UACR <30 mg/g],

**Figure 1.** *Continued.* CKD G1-2 A2-3 [eGFR  $\geq$ 60 ml/min and UACR  $\geq$ 30 mg/g] and CKD G 3-5 A1-3 [eGFR <60 ml/min]). Models were adjusted as under (B). (D) Plasmin-induced clot lysis time in a plasma pool from healthy volunteers. Plasma was incubated in the presence or absence of 10 mg/ml Zymosan (Sigma Aldrich) for 60 minutes at 37°C and subsequently mixed with tissue plasminogen activator (tPA, Hemochrom Diagnostica; 83 ng/ml), recalcified (CaCl 2 7.5 mM; Roth), and clotting was initiated with thrombin (Sigma Aldrich; 0.03  $\mu$ /ml). In some cases, plasma was preincubated for 30 minutes with the CFD inhibitor Danicopan 1  $\mu$ g/ml. (E) Plasmin-induced clot lysis time of CFD-deficient plasma (commercially available from Assay Pro) assessed by turbidity and lysis assay. Plasma was treated as under (D) with or without reconstitution of 1  $\mu$ g/ml CFD (Sigma Aldrich). (F) Plasmin-induced clot lysis time was assessed from plasma of healthy controls and patients on hemodialysis in the presence or absence of 10 mg/ml Zymosan (Sigma Aldrich). All experiments were performed as described under (D). (G) Enzyme-linked immunosorbent assay for CFD (Hycult Biotech). (H) Enzyme-linked immunosorbent assay for complement fragment Bb (Quidel Ortho). \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ . BMI, body mass index; CFD, complement factor D; HR, hazard ratio; hsCRP, high sensitivity C-reactive protein; MACE, major adverse cardiovascular events; NTproBNP, N terminal pro-B-Type natriuretic peptide; UACR, urine albumin-creatinine ratio.

G3-5 A1-3). Here, we could demonstrate that patients within the highest CFD tertile and CKD G3-5 had the highest risk of MACE with a hazard ratio of 2.71 (95% confidence interval, 2.06 to 3.60; **Figure 1C**). Given the strong link between CFD and thrombotic end points, we investigated the role of CFD on plasmin-induced clot lysis time, an established predictor of cardiovascular and thrombotic events. Zymosan-induced activation of the AP in plasma significantly prolonged clot lysis time (837 $\pm$ 99 sec versus 585 $\pm$ 74 sec,  $P < 0.01$ ). This effect was abolished by preincubation with the factor D inhibitor danicopan, normalizing lysis times to control levels (**Figure 1D**). Vice versa, reconstitution of CFD in CFD-deficient plasma significantly elevated Zymosan-induced clot lysis time (1179 $\pm$ 179 sec versus 855 $\pm$ 193 sec,  $P < 0.05$ ; **Figure 1E**). These data suggest a strong link between CFD, activation of the alternative pathway, and clot lysis time.

Next, we conducted experiments under similar conditions, comparing patients on hemodialysis ( $n=10$ ) with healthy controls ( $n=10$ ). Here, patients on hemodialysis had a significant higher clot lysis time compared with healthy controls (hemodialysis: 2743 $\pm$ 1009 sec versus controls: 1202 $\pm$ 631 sec,  $P < 0.001$ ), which was further elevated by Zymosan. Strikingly, this elevated clot lysis time in patients on hemodialysis was linked to elevated plasma levels of CFD (healthy controls 0.71 $\pm$ 0.14  $\mu$ g/ml versus hemodialysis 8.13 $\pm$ 5.27  $\mu$ g/ml,  $P = 0.003$ , **Figure 1G**) and fragment Bb (healthy controls 0.79 $\pm$ 0.16 versus hemodialysis 1.37 $\pm$ 0.67,  $P = 0.02$ , **Figure 1H**) as an indicator of a higher risk of AP activity in patients on hemodialysis.

In summary, our study suggests increased levels of CFD and AP activity in patients with CKD that trigger a pro-coagulatory, thrombotic phenotype and link complement activity to a higher risk of thrombotic event rate in CKD.

#### Disclosures

Disclosure forms, as provided by each author, are available with the online version of the article at <http://links.lww.com/JSN/F410>.

#### Author Contributions

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#### Declarative Statements

This study includes clinical experimentation and received Institutional Review Board or Ethics Committee approval from University Hospital Aachen and the UK Biobank (Application number 88924). All patients provided written informed consent. This study includes clinical experimentation and complies with the Declaration of Helsinki.

### Data Availability Statements

Data Type: Observational Data. Data belong to the UK biobank and cannot be shared before agreement of the UK biobank.

### Supplemental Material

This article contains the following supplemental material online at <http://links.lww.com/JSN/F411>.

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