

Complement Classical and Alternative Pathway Activation Contributes to Diabetic Kidney Disease

Progression: A Glomerular Proteomics on Kidney Biopsies

Yang Yang¹, Ying Zhang¹, Yuan Li¹, Xinjin Zhou², Kazuho Honda³, Dedong Kang³, Muxi Wang⁴, Jing-Hua Yang⁵, Zongping Xia⁵, Yuan Wei¹, Lu Liu¹, Ruimin Hu¹, Takashi Takaki³ & Guolan Xing^{1*}

Supplementary Material Contents

Table S1. Complement lectin pathway proteins in the glomerular proteome of DKD patients and normal controls.

Table S2. Clinical and pathologic characteristics of the validation cohort.

Table S3. Immunohistochemical scores of complement proteins in glomeruli of DKD patients and normal controls.

Table S1. Complement lectin pathway proteins in the glomerular proteome of DKD patients and normal controls

Protein Description	Accession	Abundance in controls	Abundance in DKD	Abundance in DKD of each class			
				Class I DKD	Class II DKD	Class III DKD	Class IV DKD
Mannose-binding protein C (MBL)	P11226	0	0.6	0	0.2	0.5	1.3
Collectin-10	Q9Y6Z7	0	0	0	0	0	0
Collectin-11	Q9BWP8	0	0.2	0	0.1	0.4	0.1
Ficolin-1	O00602	0	0	0	0	0	0
Ficolin-2	Q15485-1	0	1.3	0	1.9	1.9	0.5
Ficolin-3	O75636-1	0	12.1	3.5	8.9	14.9	15.4
Mannan-binding lectin serine protease 1 (MASP-1)	P48740-1	0	0	0	0	0	0
Mannan-binding lectin serine protease 2 (MASP-2)	O00187-1	0	1.0	0	0.9	1.1	1.3
Mannan-binding lectin serine protease 3 (MASP-3)	N/A	N/A	N/A	N/A	N/A	N/A	N/A
MBL-associated protein 19 (Map19)	N/A	N/A	N/A	N/A	N/A	N/A	N/A
MBL-associated protein 44 (Map44)	N/A	N/A	N/A	N/A	N/A	N/A	N/A

MASP-3 and MAp44 are splice products of the *MASP-1* gene. Map19 are generated by alternative splicing of the *MASP-2* gene. The abundances of their peptides are included in the MASP-1 and MASP-2 proteins in spectral analysis respectively. MASP-3, Map19 and Map 44 are not yet included in the Uniport database and therefore do not have protein accessions. MBL, mannose-binding lectin; MASP, MBL-associated serine protease; N/A, Not Available.

Table S2. Clinical and pathologic characteristics of the validation cohort

	DKD (<i>n</i> =20)
Age (years), mean \pm SD	53.35 \pm 8.95
Gender (male/female)	12/8
Diabetes duration (years), median (IQR)	10 (8,20)
History of hypertension, <i>n</i> (%)	18 (90%)
Fasting plasma glucose (mg/dl), mean \pm SD	177 \pm 49.34
Glycated hemoglobin (HbA1c), mean \pm SD	(7.02 \pm 1.34) %
SCr (μ mol/L), mean \pm SD	226.25 \pm 136.42
eGFR (ml/min/1.73 m ²), mean \pm SD	41.9 \pm 11.38
Proteinuria (g/24h), mean \pm SD	4.67 \pm 2.82
Hematuria, <i>n</i> (%)	1 (5%)
Serum C3 (g/L), mean \pm SD	1.21 \pm 0.29
Glomerular class (I/II/III/IV), <i>n</i>	5/5/5/5
IFTA score (0/1/2/3), <i>n</i>	0/5/4/11

SD, standard deviation; IQR, interquartile range; SCr, serum creatinine; eGFR, estimated glomerular filtration rate; C3, complement 3; IFTA, interstitial fibrosis and tubular atrophy.

Table S3. Immunohistochemical scores of complement proteins in glomeruli of DKD patients and normal controls.

	Group	Glomeruli number (<i>n</i>)	Glomerular immunohistochemical scores							
			C1q	C3	C4	C5b-9	CFB	CFH	MBL	MASP
1	NC	28	0	0	0	0	0	0	0	0
2	NC	31	0	0	0	0	0	0	0	0
3	NC	22	0	0	0	0	0	0	0	0
4	DKD I	14	0	0	1	1	0	0	0	0
5	DKD I	23	0	1	0	0	0	0	0	0
6	DKD I	12	0	0	0	0	0	0	0	0
7	DKD I	16	1	2	1	2	0	1	0	0
8	DKD I	24	0	1	0	0	0	1	0	0
9	DKD II	14	0	1	1	1	1	1	0	0
10	DKD II	15	0	1	1	2	0	1	0	0
11	DKD II	26	0	2	2	1	0	0	0	0
12	DKD II	12	1	3	1	2	1	1	0	0
13	DKD II	17	0	3	2	2	1	2	0	0
14	DKD III	18	1	3	2	3	2	1	0	0
15	DKD III	21	3	3	3	2	2	1	0	0
16	DKD III	15	1	2	3	2	1	2	0	0
17	DKD III	24	1	3	2	1	1	1	0	0
18	DKD III	18	1	2	2	2	1	1	0	0
19	DKD IV	17	1	2	2	2	2	2	0	0
20	DKD IV	16	1	3	3	2	1	1	0	0
21	DKD IV	12	1	2	3	3	1	1	0	0
22	DKD IV	18	2	3	3	2	2	2	0	0
23	DKD IV	13	2	2	3	3	1	3	0	0

The extent of glomerular staining for complement proteins was evaluated at $\times 400$ magnification and scored semiquantitatively as follows: 0, no staining; 1, weak and spotty intraglomerular staining; 2, moderate and segmental intraglomerular staining; and 3, strong and diffuse intraglomerular staining. NC, normal controls; DKD, diabetic kidney disease; IHC, immunohistochemistry.