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SHORT COMMUNICATION

Complement 7 Is Up-Regulated in Human Early Diabetic Kidney Disease

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There is a temporal window from the time diabetes is diagnosed to the appearance of overt kidney disease during which time the disease progresses quietly without detection. Currently, there is no way to detect early diabetic nephropathy (EDN). Herein, we performed an unbiased assessment of gene-expression analysis of postmortem human kidneys to identify candidate genes that may contribute to EDN. We then studied one of the most promising differentially expressed genes in both kidney tissue and blood samples. Differential transcriptome analysis of EDN kidneys and matched nondiabetic controls showed alterations in five canonical pathways, and among them the complement pathway was the most significantly altered. One specific complement pathway gene, complement 7 (*C7*), was significantly elevated in EDN kidney. Real-time PCR confirmed more than a twofold increase of *C7* expression in EDN kidneys compared with controls. Changes in C7 gene product level were confirmed by immunohistochemistry. C7 protein levels were elevated in proximal tubules of EDN kidneys. Serum C7 protein levels were also measured in EDN and control donors. C7 levels were significantly higher in EDN serum than control serum. This latter finding was independently confirmed in a second set of blood samples from a previously collected data set. Together, our data suggest that *C7* is associated with EDN, and can be used as a molecular target for detection and/or treatment of EDN. *(Am J Pathol 2018, 188: 2147–2154; https://doi.org/10.1016/j.ajpath.2018.06.018)*

Over the past two decades, the incidence of end-stage renal disease in the United States has nearly doubled because of a steady increase in diabetic nephropathy (DN). In 1982, only 23% of new end-stage renal disease cases were attributed to diabetes, but by 1999, at 43%, DN became the leading cause of end-stage renal disease.[1] In 2015, the incidence of end-stage renal disease was >130,000, with prevalence >700,000 and an annual death rate of >100,000 individuals.[2] Despite progress made in delaying dialysis dependence, these trends are expected to increase, at least for the near future.

DN affects 15% to 25% of type 1 diabetics and 30% to 40% of type 2 diabetics.[3] The detection of albumin in urine is often the first clinical indication of kidney disease. The natural progression of diabetic kidney disease has been closely examined in type 1 diabetic patients, in whom timing of disease onset is generally known. Investigation into type 1 diabetes has shown progressive decline in renal function over time (>3.5 mL/minute per year).[4] However, for many years, the decline was not detected by standard laboratory testing because patients were deemed to have normal renal function if the estimated glomerular filtration rate was >60 mL/

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minute. Later in the disease process, individual patients can have differing rates of renal decline. Progressive renal decline precedes the onset of microalbuminuria and, as it continues, it increases the risk of proteinuria.[4]

Evidence from autopsy studies indicates that diabetic nephropathy remains relatively underdiagnosed,[5–7] and diabetic kidney damage,[8] often with no overt clinical findings, is usually confirmed during postmortem pathologic examination. In 2010, the Renal Pathology Society developed a new classification system for diabetic kidney disease.[9] According to this classification: class I kidneys show increased thickness of glomerular basement membrane (≥395 nm in females and ≥430 nm in males), class II kidneys have mild (class IIa) to severe (class IIb) mesangial expansion, class III kidneys show at least one convincing Kimmelstiel-Wilson lesion, and in class IV kidneys show 50% of glomeruli have global glomerular sclerosis. Pathologic staging of diabetic kidney disease appears to be unrelated to clinical staging of chronic kidney disease, such as blood creatinine level.

Mechanisms underlying early diabetic nephropathy remain unknown, and there is a paucity of literature on renal molecular targets in early DN (EDN).[10–16] Most differential gene expression studies have been performed in rodent diabetic models, and although rodent experiments provide important insights into disease mechanisms, they are limited because of the inherent differences between murine and human diabetic kidney disease. In the few human studies that have been reported in the literature, all have looked at kidneys with advanced diabetic kidney disease. There appears to be no study in the literature that has investigated gene expression in postmortem human kidneys with early diabetic nephropathy, otherwise scheduled for the kidney donor pool. Herein, we have compared transcriptome profiles of human postmortem early diabetic kidneys with those of nondiabetic kidneys. We then selected one of the most significantly increased EDN genes for further examination in postmortem human kidney and blood.

Materials and Methods

This study was approved by the Massachusetts General Hospital Institutional Review Board and abided by the guidelines set forth by the Declaration of Helsinki. No donor organs were obtained from executed prisoners or other institutionalized individuals at any national transplant center. Organ procurement offices were coordinated to retrieve organs with appropriate informed consents. All patient information was deidentified and was thus Health Insurance Portability and Accountability Act compliant.

Study Population and Sample Collection

Postmortem biological samples (blood and kidney tissue) from nondiabetics and individuals with EDN were obtained

Table 1 Summary of Patient Demographics

Sample type	Nondiabetic	Diabetic
Harvested kidneys		
n (%)	18 (51)	17 (49)
Male sex, *n* (%)	8 (44)	6 (35)
Age in years, mean (range)	50 (17–75)	57 (34–78)
Hypertension, *n* (%)	9 (50)	17 (100)
Obese, *n* (%)	5 (28)	12 (71)
Tobacco use, *n* (%)	12 (67)	10 (59)
White, *n* (%)	14 (78)	12 (71)
Serum creatinine, mean (range), mg/dL*	1.4 (0.4–4.5)	1.3 (0.5–2.3)
Proteinuria (≥1 + by UA), *n* %	4 (22)	7 (41)
Hemoglobin A1c, mean % (range)	5.4 (4.7–5.9)	8.2 (6.5–11.3)
eGFR (per MDRD), mean mL/minute	69.1	51.6
Stage I to IIIA eGFR, *n* %†	11 (61)	9 (53)
Serum complement C3, mg/dL	112	137
Serum complement C4, mg/dL	25	29
Total complement CH50, U/mL	51	57
Alternative complement AH50, U/mL	97	127
Kidney samples for microarray‡		
n	4	4
Male sex, *n*	2	2
Age in years, mean (range)	52 (24–65)	64 (55–78)
Hypertension, *n* (%)	3 (75)	4 (100)
BMI in kg/m^2, mean (range)	27 (26–29)	35 (22–43)
Tobacco use, *n* (%)	2 (50)	2 (50)
White, *n* (%)	3 (75)	3 (75)
Terminal serum creatinine, mean, mg/dL	0.9	1.4
Proteinuria (≥1 + by UA), %	50	50
Hemoglobin A1c, mean, %	5.4	9.5
eGFR, mean mL/minute	>60	>60
PRIMO blood samples		
n	20	39
Male sex, *n* (%)	14 (70)	30 (77)
Age in years, mean (range)	66 (47–81)	66 (48–84)
Hypertension, *n* (%)	19 (95)	38 (97)
Obese, *n* (%)	6 (30)	24 (62)
Tobacco use, *n* (%)	11 (55)	23 (59)
White, *n* (%)	15 (75)	29 (74)
Serum creatinine, mean, mg/dL*	2.1	2.1
Proteinuria (≥1 + by UA), *n* (%)	10 (50)	24 (62)
eGFR (per MDRD), mean mL/minute	33	34
Class I to IIIA or better eGFR, *n* (%)†	2 (10)	5 (13)

*At time of admission.
†Based on MDRD equations.
‡Subset of harvested kidneys.
AH50, alternative pathway complement activity; BMI, body mass index; CH50, total complement activity; eGFR, estimated glomerular filtration rate; MDRD, Modification of Diet in Renal Disease; PRIMO, Paricalcitol Capsules Benefits Renal Failure Induced Cardiac Morbidity in Subjects with Chronic Kidney Disease Stage 3/4; UA, urinalysis.