Supplementary information
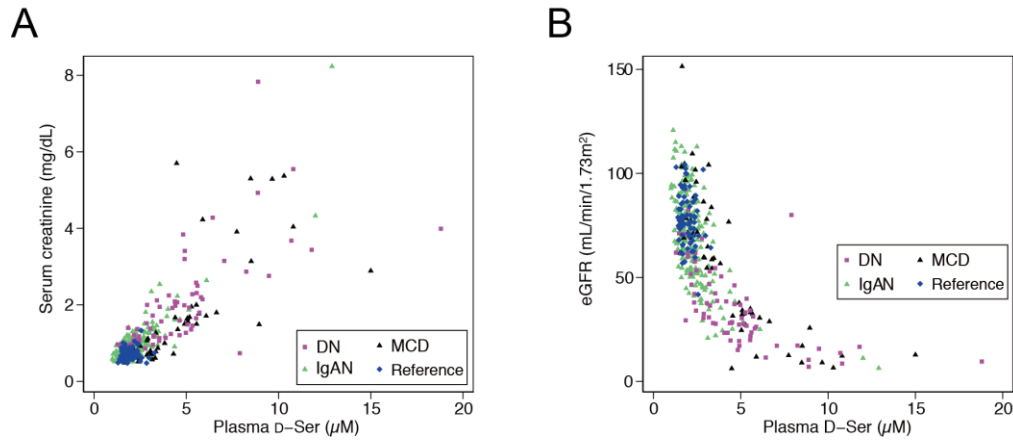
Identification of diabetic nephropathy in patients undergoing kidney biopsy through blood and urinary profiles of D-serine

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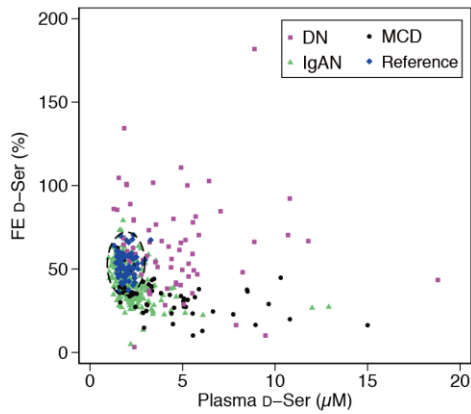
5 Supplementary figures
3 Supplementary tables
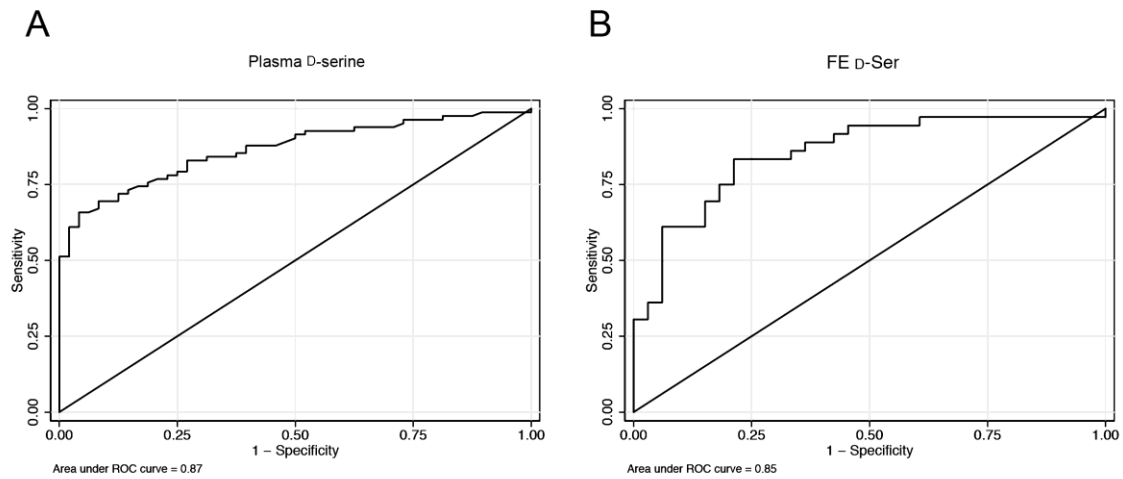
Supplementary Figure 1. Plasma level of D-serine and kidney function. (A and B) Scatter plot of plasma level of D-serine with (A) serum level of creatinine and (B) estimated glomerular filtration rate (eGFR). Values are shown without logarithm transformation. DN, diabetic nephropathy; MCD, minimal change disease; IgAN, IgA nephropathy; reference, non-kidney disease participants.
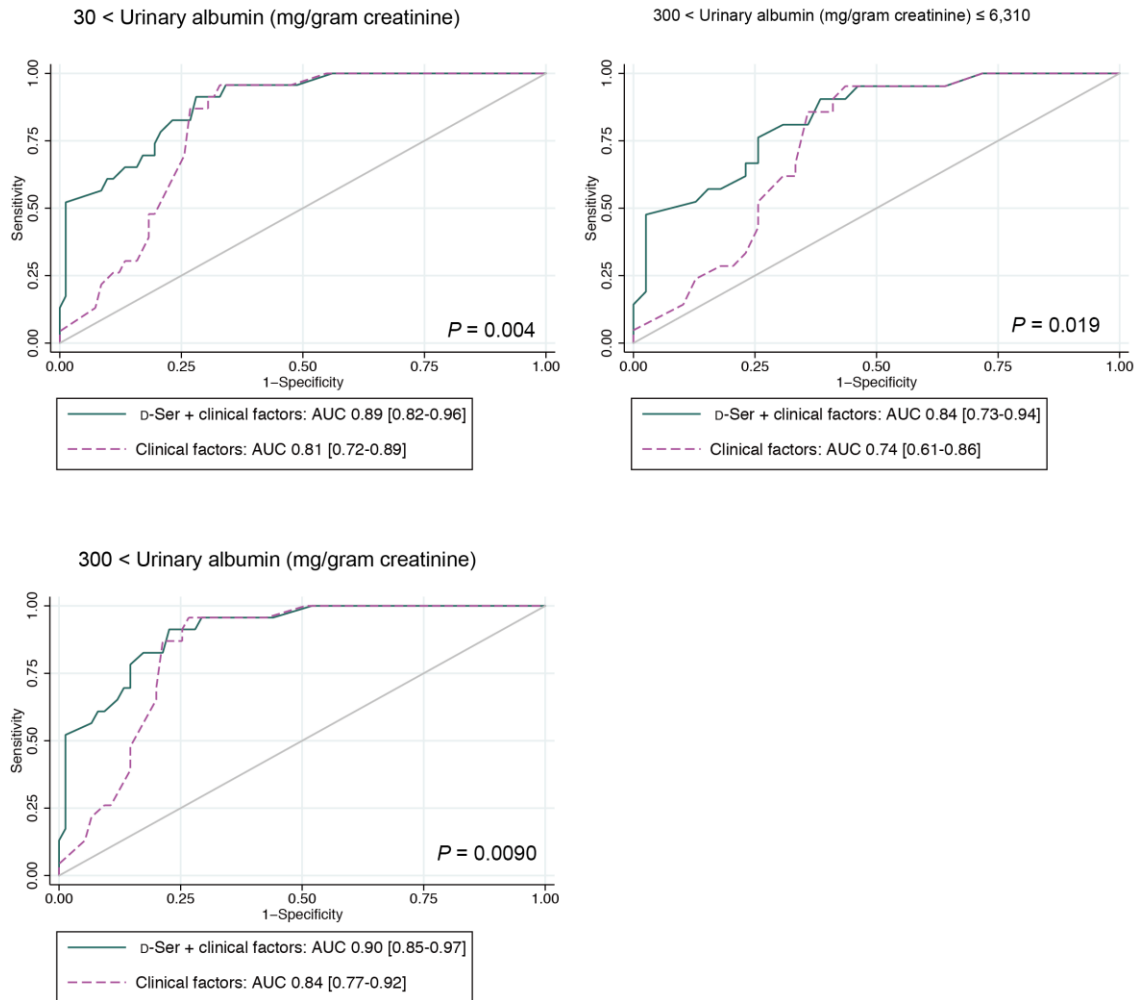
Supplementary Figure 2. Scatter plots of plasma level and fractional excretion (FE) of D-serine in patients with all participants. Values are shown without logarithm transformation. The eclipse represents 95% confidence interval of reference (non-kidney disease participants). DN, diabetic nephropathy; MCD, minimal change disease; IgAN, IgA nephropathy.

A

Plasma D-serine

B

FE D-Ser

Supplementary Figure 3. Detection of diabetic nephropathy (DN) using profiles of D-serine in participants without IgA nephropathy. (A) Receiver operating characteristic (ROC) curve analysis of plasma level of D-serine in eliminating reference (non-kidney disease participants) from kidney diseases in prediction dataset. (B) ROC curve analysis of urinary fractional excretion (FE) of D-serine in separating DN from other diseases in prediction dataset with the plasma level of D-serine above 2.34 μM.

Supplementary Figure 4. Combination of D-serine and clinical factors for the detection of diabetic nephropathy. Receiver operating characteristic (ROC) curve analysis of plasma-high and fractional excretion (FE)-high profile of D-serine with clinical factors (age, sex, estimated glomerular filtration rate [eGFR], and presence of albuminuria) in validation dataset of several ranges of albuminuria.

Supplementary Figure 5. Relation of fractional excretion (FE) of D-serine and clinical parameters in patients with plasma level of D-serine above 2.34 μM. (A and B) Scatter plots for FE of D-serine with (A) β2-microglobulin (β2-MG) and (B) N-acetyl-β-D-glucosaminidase (NAG) were shown in participants with or without diabetic nephropathy (DN), or in all participants. MCD, minimal change disease; IgAN, IgA nephropathy; reference, non-kidney disease participants. Values are transformed to common logarithm.

Table S1. Baseline characteristics of the study.

	Diabetic nephropathy (n = 69)			Minimal change disease (n = 53)			IgA nephropathy (n = 194)			Reference (n = 72)		
Serum sodium, mEq/L	140	(138	- 141)	139	(138	- 141)	140	(139	- 141)	140	(138	- 141)
Serum potassium, mEq/L	4.1	(3.8	- 4.4)	4.0	(3.8	- 4.4)	4.1	(3.9	- 4.3)	4.0	(3.8	- 4.2)
Serum chloride, mEq/L	107	(102	- 108)	105	(103	- 109)	105	(104	- 107)	103	(103	- 105)
Serum adjusted calcium, mg/dL	9.4	(9.1	- 9.8)	9.8	(9.6	- 10.1)	9.3	(9.1	- 9.5)	9.2	(9.1	- 9.4)
Serum inorganic phosphate, mg/dL	3.7	(3.1	- 4.2)	4.0	(3.5	- 4.3)	3.3	(3.0	- 3.7)	3.4	(3.1	- 3.6)
Serum AST, U/L	21.0	(16.0	- 32.0)	23.0	(18.0	- 32.0)	20.0	(17.0	- 23.0)	19.5	(16.8	- 22.3)
Serum ALT, U/L	18.0	(11.0	- 26.0)	17.0	(12.0	- 24.0)	17.0	(12.0	- 23.0)	15.5	(12.0	- 20.3)
Serum total cholesterol, mg/dL	190	(162	- 224)	339	(278	- 450)	198	(179	- 218)	192	(169	- 214)
Serum triglyceride, mg/dL	156	(128	- 207)	195	(143	- 233)	107	(76	- 162)	90	(67	- 125)
Serum LDL, mg/dL	107	(87	- 129)	213	(161	- 292)	112	(94	- 134)	115	(95	- 127)
Serum LDH, U/L	224	(196	- 250)	230	(202	- 275)	185	(159	- 208)	159	(14	- 176)
Serum IgG, mg/dL	1028	(797	- 1219)	526	(432	- 673)	1082	(848	- 1302)	1110	(947	- 1184)
Serum IgA, mg/dL	272	(224	- 407)	265	(206	- 327)	303	(236	- 401)	190	(152	- 246)
Serum IgM, mg/dL	83	(51	- 123)	86	(65	- 145)	93	(66	- 135)	64	(41	- 100)
Serum C3, mg/dL	110.3	(99.3	- 123.8)	129.0	(112.0	- 149.0)	101.5	(90.0	- 117.8)	100.0	(90.8	- 113.0)
Serum C4, mg/dL	30.3	(25.9	- 34.8)	37.0	(30.0	- 44.0)	25.9	(21.0	- 30.7)	24.0	(19.0	- 28.3)
Blood white blood cell, ×10^3 /μL	6.7	(5.2	- 8.1)	5.8	(5.0	- 7.5)	6.6	(5.4	- 7.5)	5.6	(4.7	- 6.4)
Blood hemoglobin, g/dL	11.8	(10.6	- 13.6)	13.1	(11.8	- 14.1)	13.4	(12.2	- 14.5)	13.7	(12.5	- 14.8)
Blood platelet ×10^3/μL	225	(183.0	- 277.0)	255	(205.0	- 290.0)	240	(205.0	- 274.5)	223	(192.7	- 272.5)
Blood CRP mg/dL	0.08	(0.04	- 0.24)	0.09	(0.02	- 0.45)	0.04	(0.01	- 0.12)	0.06	(0.04	- 0.11)

Values are described as median (IQR). Reference, non-kidney disease participants; AST, aspartate aminotransferase; ALT, alanine aminotransferase; LDL, low density lipoprotein; LDH, lactate dehydrogenase; CRP, C-reactive protein.

Table S2. Characteristics of prediction and validation data.

	Prediction data (n = 259)					Validation data (n = 129)					P
Serum sodium, mEq/L	140	(	139	-	141	)	140	(	139	-	141	)	0.628
Serum potassium, mEq/L	4.1	(	3.9	-	4.4	)	4.1	(	3.8	-	4.3	)	0.137
Serum chloride, mEq/L	105	(	103	-	107	)	105	(	103	-	107	)	0.452
Serum adjusted calcium, mg/dL	9.3	(	9.1	-	9.6	)	9.3	(	9.1	-	9.7	)	0.231
Serum inorganic phosphate, mg/dL	3.4	(	3.1	-	3.8	)	3.5	(	3.1	-	4.0	)	0.439
Serum AST, U/L	20.0	(	17.0	-	25.0	)	20.0	(	17.0	-	26.0	)	0.773
Serum ALT, U/L	17.0	(	12.0	-	23.0	)	17.0	(	12.0	-	24.0	)	0.707
Serum total cholesterol, mg/dL	202	(	179	-	230	)	204	(	178	-	254	)	0.457
Serum triglyceride, mg/dL	123	(	82	-	190	)	120	(	82	-	187	)	0.694
Serum LDL, mg/dL	116	(	95	-	144	)	120	(	95	-	150	)	0.584
Serum LDH, U/L	186	(	161	-	221	)	193	(	164	-	237	)	0.260
Serum IgG, mg/dL	1024	(	769	-	1228	)	1005	(	768	-	1200	)	0.563
Serum IgA, mg/dL	274	(	216	-	371	)	253	(	191	-	356	)	0.516
Serum IgM, mg/dL	83	(	57	-	125	)	93	(	59	-	129	)	0.875
Serum C3, mg/dL	107.0	(	93.2	-	123.5	)	105.8	(	93.0	-	119.7	)	0.901
Serum C4, mg/dL	27.0	(	22.4	-	33.5	)	27.0	(	21.3	-	34.8	)	0.639
Blood white blood cell, ×10^3 /μL	6.9	(	5.3	-	8.8	)	6.8	(	5.9	-	8.6	)	0.600
Blood hemoglobin, g/dL	13.1	(	12.1	-	14.5	)	13.5	(	12.3	-	14.4	)	0.679
Blood platelet ×10^3/μL	233	(	170	-	268	)	227	(	161	-	270	)	0.755
Blood CRP mg/dL	0.07	(	0.03	-	0.16	)	0.07	(	0.02	-	0.11	)	0.704

Values are described as median (IQR). AST, aspartate aminotransferase; ALT, alanine aminotransferase; LDL, low density lipoprotein; LDH, lactate dehydrogenase; CRP, C-reactive protein.

Table S3. Logistic regression analysis of D-serine profile in the detection of diabetic nephropathy (DN).

Variables		Unadjusted odds ratio					P	Adjusted odds ratio						P
Independent	Dependent													
Plasma-high & FE-high D-Ser profile	DN	24.8	(	11.1	-	55.2	)	<0.001	14.7	(	6.0	-	36.1	)	<0.001

Analysis was performed with the prediction data. Odds ratios were adjusted for age over 60 years, sex, urinary albumin level per 1 g/gram creatinine, and estimated glomerular filtration rate over 60 mL/min/1.73 m^2.