IgG Subclass Expression in Diabetic Nephropathy

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Abstract

Background: This study aimed to analyze the distribution of IgG subclasses in diabetic nephropathy (DN) and its association with clinico-pathological features.

Methods: Forty DN cases were analyzed to identify IgG subclasses, as well as collagen IV α5, CD34, and KIM-1.

Results: Both IgG and its subclasses showed a linear expression and overlapped with collagen IV α5 on glomerular basement membrane (GBM) and some of tubular basement membrane (TBM), without complement deposition. Eleven cases of IgG subclass deposition along both GBM and TBM were associated with more proteinuria. Five cases of TBM-only IgG subclass deposition were accompanied with less KIM-1 positivity and more arteriosclerosis. The major IgG subclasses expressed on GBM were IgG1 and IgG2, while TBM expression was mainly IgG1 and IgG3. Glomerular IgG1-positive status was associated with less CD34 expression, while IgG2-positive status was associated with thicker GBM. Expression of multiple IgG subclasses along TBM showed less KIM-1 positivity and interstitial inflammation than those with isotype or no IgG subclass expression.

Conclusions: IgG subclasses were selectively deposited along GBM and TBM in DN, which was determined by their profiles and severity of glomerular/tubular injury. IgG and its subclass deposition is not causal, but the consequence of renal injury and these positive statuses are associated with different DN injuries.

Introduction

Diabetic nephropathy (DN) has a unique histological pattern, including glomerular basement membrane (GBM) thickening, mesangial expansion, and glomerulosclerosis, among others [1]. As a nonimmunological-related renal disease, the detection of IgG deposition along GBM and/or tubular basement membrane (TBM) in some DN cases is surprising [2]. This linear pattern can not be detected by regular electron microscopy in DN, which is different from its deposition in immune complex-related glomerular diseases, such as membranous nephropathy (MN) [2]. Some hypotheses suggest that structural changes of the basement membrane lead to entrapment of serum proteins, including albumin and IgG [3, 4]. One study demonstrated that up to 51.5% of the DN biopsies were IgG-positive, and the IgG intensity was associated with the progression of renal injury [2].

IgG has four subclasses (IgG1-G4), each subclass has a unique profile regarding half-life, antigen binding, immune complex formation, complement activation, and triggering of effector cells. Oxelius VA found that serum IgG2 and IgG3 levels declined, while IgG1 and IgG4 were relatively normal in juvenile diabetes mellitus cases. Susanna M et al. found that IgG4 was selectively eliminated and urinary IgG4 could be a useful marker for preclinical stages of diabetic nephropathy [5, 6]. Using the biopsy tissue, Hemminger J et al. conducted a large retrospective study of IgG subclasses in 1084 cases, which showed that IgG4-dominant/codominant deposition with PLA2R-positive status was associated with primary MN, while

IgG1 dominant/codominant with weak or absent IgG4 deposition was associated with autoimmune disease-related MN [7]. However, no IgG subclass in DN was included in this study. In 1984, Melvin T et al. studied IgG subclass in nine DN cases, and found that only IgG4 had the same glomerular linear deposition as IgG [8]. This is difficult to explain by using the IgG profiles, since IgG4 has an anionic charge and the lowest serum concentration compared with the other subclasses, and GBM is anionically charged [6, 9]. Further, the effect of tubular IgG deposition remains to be studied.

Therefore, we studied the distribution of IgG and its subclasses in 40 DN cases, and found more proteinuria in cases with both GBM and TBM IgG subclass deposition than negative ones. Less tubular injuries, but more arteriosclerosis were detected in TBM-only deposition group. There were more DN cases of GBM linear staining with the IgG1 or IgG2 isotype than those with the IgG4 isotype. Glomerular IgG1-positive status was associated with least glomerular epithelial cell injury, while IgG2-positive status was associated with thicker GBM compared to other groups. Furthermore, DN with multiple IgG subclass expression alon TBM showed less tubular injury and interstitial inflammation than those with isotype IgG subclass expression.

Materials And Methods

Patients

Among 125 patients with T2DM and biopsy-proven DN between August 2017 and July 2019 at the Hangzhou Hospital of Traditional Chinese Medicine, 84 cases showed an IgG linear pattern by immunofluorescence (IF). Forty cases were enrolled according to the following inclusion criteria: (1) T2DM; (2) a diagnosis of DN proven by kidney biopsy; (3) IF showed IgG-positive status; and (4) Four IgG subclasses could be fully applied. The exclusion criteria were: (1) coexistence of nondiabetic renal diseases, such as MN, or system diseases; and (2) absence of glomeruli or global sclerosis in IF specimens. The study was approved by the ethical committees of Hangzhou Hospital of Traditional Chinese Medicine.

Clinical and pathologic characteristics

The following clinical information was collected: age, gender, 24-hour proteinuria, serum albumin, serum creatinine (Scr), and the estimated glomerular filtration rate (eGFR).

All specimens were processed for light microscopy (LM), IF, immunohistochemistry (IHC), and electron microscopy (EM). Classification of DN and histological scoring were done according to the criteria reported by Tervaert et al [1]. Interstitial fibrosis and tubular atrophy (IFTA, 0–3 score), as well as arteria hyalinosis and sclerosis (0–3 score) were scored according to methods described by the previous study [1]. Diagnosis, classification, and the score of these pathological findings were evaluated and confirmed by two renal pathologists. GBM and TBM thickness were measured under an electron microscope according to studies reported by Haas and Tyagi I [10, 11]. Podocyte effacement was measured according to the degree of foot process fusion [12].

Immunofluorescence and immunohistochemistry staining

Frozen tissues were used for IgG (#F0202; 1:50, DAKO, Denmark) and IgG subclass (IgG1-IgG4, #F0767, #F4516, #F4641, #F9890, 1:50, Sigma-Aldrich, USA) staining by direct IF. Double staining of IgG or IgG subclass with anti-human collagen IV α5 (#C-452, 1:100, Cosmo corporation, Japan) by indirect IF (AF594 of Donkey anti-rat IgG as secondary antibody, 1:100,)was also performed. An Olympus BX53 fluorescence microscope (Japan) was used to analyze the IF slides.

Immunohistochemistry for KIM-1 (#14971, 1:200, Cell Signaling Technology, USA) and CD34 (ab81289, 1:100 titer, Abcam, Britain) was conducted using a Ventana BenchMark XT system. KIM-1 intensity was scored as follows: 0 = none, 1 = weakly positive, 2 = positive, and 3 = strong positive. CD34- and KIM-1-positive statuses were calculated as the percentage of positive area per glomerulus or cortex, respectively [13, 14].

Statistical analysis

Statistical analysis was performed with SPSS 17.0. Normally distributed data were expressed as mean ± SD. A comparison of clinical and pathological characteristics among groups was assessed by t-test or ANOVA for continuous variables, and by nonparametric tests for discontinuous variables. Categorical variables were expressed as percentages and comparisons among groups, which were assessed by chi-square test or the Fisher's exact test. A P-value of less than 0.05 was considered significant.

Results

Clinical and morphological features

The average age at the time of renal biopsy of all 40 cases was 55.6 ± 15.7 years, and 28 cases comprised males. Patients showed proteinuria (urinary protein 4.8 ± 3.2 g/day), with normal to mild reduction of renal function (Scr 114.2 ± 61.0 umol/L, eGFR 78.4 ± 38.8 ml/min per 1.73 m^2) and serum albumin (30.4 ± 7.2 g/L). Renal biopsy showed seventy-five percent of DN cases were in stage three. The average percentage of glomerulosclerosis was 17.5 ± 14.8%, and the average percentage of podocyte effacement was 76.6 ± 15.4%. IFTA score was 2.0 ± 0.8. All cases had minor to moderate linear IgG expression along the GBM, and IgG colocalized with collagen IV α5 staining (Fig. 1A). Thirty-one cases showed minor linear IgG along TBM (Fig. 1A). All cases showed negative or trace complements (C3, C4, C1q) along GBM or TBM (Fig. 1B).

Association between IgG subclass location and clinicopathological features

IgG subclasses were stained with the similar intensity of IgG, and the glomerular positive expression was also colocalized with collagen IV α5 expression (Fig. 1C). Positive IgG but negative or trace expression of IgG subclass (the None group) was observed in 11 cases. Thirteen cases showed IgG subclass

expression along GBM only (GBM-only group), while five cases were positive along TBM only (TBM-only group). The remaining 11 cases had positive expression on both GBM and TBM (the Both group) (Table 1).

Table 1
Clinical-pathological correlations among different groups based on IgG subclass locations

	None (n = 11)	GBM-only (n = 13)	TBM-only (n = 5)	Both (n = 11)
Male (%)	9.1	54.2	75	63.6
Age (year)	55.1 ± 9.8	**51.4 ± 12.6** [a]	54.6 ± 11.3	61.6 ± 7.2
Proteinuria(g/24 h)	3.4 ± 2.2	5.4 ± 4.6	4.5 ± 1.9	**5.7 ± 2.2** [b]
Albumin (g/L)	33.4 ± 7.7	30.2 ± 7.6	**23.5 ± 3.6** [a,b]	30.7 ± 5.8
Scr (μmol/L)	124.6 ± 82.6	105.2 ± 43.7	139.3 ± 84.6	103.1 ± 44.7
eGFR(ml/min/1.73 m^2)	75.2 ± 43.9	86.0 ± 37.7	64.1 ± 45.7	78.8 ± 35.4
DN stage □ (%)	18.2	15.4	0	18.2
DN stage □ (%)	72.7	76.9	100.0	81.8
GS(%)	17.6 ± 11.5	15.3 ± 15.7	9.1 ± 8.6	23.6 ± 17.6
CD34 + area (%)	33.6 ± 5.6	31.1 ± 7.7	33.5 ± 7.5	28.5 ± 7.9
GBM thickness (nm)	667.5 ± 131.8	732.0 ± 180.5	704.0 ± 185.4	645.1 ± 165.1
Podocyte effacement	68.6 ± 17.7	82.8 ± 12.5	82.5 ± 9.6	73.8 ± 16.9
IFTA score	1.9 ± 0.7	2.1 ± 0.8	2.0 ± 1.2	2.1 ± 0.7
KIM-1 intensity	2.5 ± 0.4	2.6 ± 0.5	**1.4 ± 0.9** [a,b,c]	2.6 ± 0.6
KIM-1 + area (%)	27.7 ± 11.3	27.7 ± 15.6	**13.0 ± 9.7** [a,b,c]	32.0 ± 13.1
TBM thickness (nm)	1112.0 ± 361.0	1285.5 ± 601.9	1184.0 ± 434.6	1257.0 ± 512.3
Inter-infla score	1.9 ± 0.7	2.1 ± 0.8	1.8 ± 1.1	1.8 ± 0.8
Hyalinosis score	2.0 ± 0.0	1.7 ± 0.8	1.8 ± 0.6	1.8 ± 0.4
A-sclerosis score	0.6 ± 0.5	0.5 ± 0.5	**1.2 ± 0.4** [b,c]	0.7 ± 0.5

Abbreviations: GS: Glomerular sclerosis; CD34 + area (%): CD34 positive area per glomeruli; IFTA: interstitial fibrosis and tubular atrophy, KIM-1 + area (%): KIM-1 positive area per cortex; KIM intensity (0–3 score); Inter-infla: interstitial inflammation; A-sclerosis score: Arteriosclerosis score.

A, vs the “Both” group, P < 0.05; b, vs the “None” group, P < 0.05; c, vs the GBM-only group, P < 0.05;

Among these four groups, higher proteinuria was detected in the Both group than the other groups, thereby indicating more glomerular injury. The TBM-only group showed less proteinuria and KIM-1 expression than other groups, thereby indicating less tubular injury. However, its arteriosclerosis score, a vascular injury marker, was higher than the None and the GBM-only groups (Table 1).

Association between GBM IgG subclass deposition and clinicopathological features

Twenty-four of the forty cases showed different IgG subclass expression on GBM, including thirteen cases from the GBM-only group and eleven cases from the Both group (Table 1). To determine whether glomerular IgG subclass was correlated with glomerular and tubulointerstitial injury, all forty cases were divided into six groups according to the positive status of IgG subclass expression on glomeruli: sixteen cases with trace to minor staining of IgG subclass (the None group); eight cases with IgG1- or IgG2-positive status, respectively (IgG1-only or IgG2-only group); one case with IgG3-positive (IgG3-only group) status; three cases with IgG4-positive (IgG4-only group) status, and four cases with ≥ 2 IgG subclass expression (the Mixed group) (Table 2 and Fig. 2A).

Table 2
Clinical-pathological findings of IgG subclass expressed on GBM

	None (n = 16)	IgG1-only (n = 8)	IgG2-only (n = 8)	IgG3-only (n = 1)	IgG4-only (n = 3)	Mixed (n = 4)
Male (%)	93.8	**37.5** [a]	**50** [a]	100	**33.3** [a]	100
Age (year)	54.9 ± 9.9	56.9 ± 9.3	57.3 ± 12.9	54	57.3 ± 17.2	51.5 ± 13.8
Proteinuria(g/24 h)	3.8 ± 2.1	5.3 ± 2.8	5.4 ± 4.8	4.9	7.3 ± 0.8	5.0 ± 5.3
Albumin (g/L)	30.3 ± 8.1	31.0 ± 5.8	29.8 ± 8.2	38.5	25.4 ± 1.9	32.0 ± 7.5
Scr(μmol/L)	129.5 ± 80.5	111.5 ± 50.2	98.2 ± 35.9	118.0	72.7 ± 17.8	120.3 ± 32.7
eGFR (ml/min/1.73 m^2)	71.5 ± 43.2	82.8 ± 42.6	83.0 ± 42.4	77.2	106.7 ± 4.2	65.2 ± 23.1
DN stage □ (%)	25	12.5	12.5	100	0	25
DN stage □ (%)	75	75	87.5	0	100	75
IgG intensity	1.8 ± 0.6	2.1 ± 0.6	**1.6 ± 0.4** [b,c]	3.0	1.7 ± 0.8	2.4 ± 0.5
C3 intensity	0.5 ± 0.5	0.1 ± 0.4	0.3 ± 0.5	0.0	0.3 ± 0.6	0.5 ± 0.6
GS(%)	14.9 ± 11.2	15.8 ± 14.2	23.1 ± 15.3	63	8.3 ± 7.2	15.1 ± 16.0
CD34 + area (%)	33.6 ± 6.0	**27.4 ± 7.6**[a]	29.6 ± 8.7	39.6	27.2 ± 4.8	29.6 ± 8.7
GBM thickness (nm)	660.0 ± 119.3	645.2 ± 121.2	**801.4 ± 184.3** [a,d]	440	553.3 ± 97.1	730.0 ± 151.0
Podocyte effacement (%)	73.6 ± 16.3	75.0 ± 17.3	82.9 ± 13.8	90	77.5 ± 14.2	75.0 ± 21.2
IFTA score	1.9 ± 0.9	1.9 ± 0.8	2.4 ± 0.7	2.0	1.7 ± 0.6	2.3 ± 0.5
KIM-1 intensity	2.2 ± 0.8	2.6 ± 0.7	2.8 ± 0.4	2.0	2.3 ± 0.3	2.7 ± 0.6
KIM-1 + area (%)	23.1 ± 12.6	27.5 ± 12.8	26.0 ± 20.2	30.0	**40.0 ± 10.0** [a]	33.3 ± 5.8
TBM thickness (nm)	1136.0 ± 372.8	1378.0 ± 517.1	1329.2 ± 699.7	600.0	1133.3 ± 273.0	1297.5 ± 500.0
Inter-infla score	1.9 ± 0.8	1.9 ± 0.8	2.3 ± 0.9	2.0	1.3 ± 0.6	2.0 ± 0.0

Abbreviations: same as Table 1.

a, *vs.* the “None” group, $P < 0.05$. b, *vs.* mixed group, $P < 0.05$. c, *vs.* IgG1-only group. d, *vs.* IgG4-only.

	None (n = 16)	IgG1-only (n = 8)	IgG2-only (n = 8)	IgG3-only (n = 1)	IgG4-only (n = 3)	Mixed (n = 4)
Hyalinosis score	1.9 ± 0.3	1.6 ± 0.7	1.6 ± 0.7	2.0	2.0 ± 0.0	2.0 ± 0.0
A-sclerosis score	0.8 ± 0.5	0.6 ± 0.5	0.7 ± 0.5	2.0	0.7 ± 0.6	0.5 ± 0.6
Abbreviations: same as Table 1.						
a, *vs.* the “None” group, P < 0.05. b, *vs.* mixed group, P < 0.05. c, *vs.* IgG1-only group. d, *vs.* IgG4-only.						

IgG1 or IgG2, but not IgG4, are more common in GBM linear staining in DN. A higher percentage of female patients were detected in IgG1-only, IgG2-only, and IgG4-only group than the None group. IgG1-only group showed the least glomerular CD34-positive status, while the IgG2-only group showed thickest GBM among the groups. However, podocyte effacement was similar among all groups. IgG4-only group showed more tubular KIM-1 staining than the None group (Table 2 and Fig. 2B).

Association between TBM IgG subclass deposition and clinicopathological features

Sixteen of the forty cases showed different IgG subclass expression on TBM, including five cases from the TBM-only group and eleven cases from the Both group (Table 1). To determine whether tubular IgG subclass was correlated with tubulointerstitial injury, all forty cases were divided into three groups according to the positive status of IgG subclass on tubules: twenty-four cases with trace to minor staining of IgG subclass (the None group); ten cases with one IgG subclass expression (the Isotype group); six cases with ≥ 2 IgG subclass expression (the Multiple types group) (Table 3). IgG1 and IgG3 were major positive subclasses along TBM in the Isotype group (n = 4, respectively, Fig. 3A).

Table 3
Tubular interstitial injuries comparing different IgG subclass categories expressed on TBM

	None (n = 24)	Isotype (n = 10)	Multiple types (n = 6)
IFTA score	2.0 ± 0.7	2.3 ± 0.7	1.7 ± 1.0
KIM-1 density	2.6 ± 0.4	2.4 ± 0.7	**1.8 ± 1.1** [a]
KIM-1 + area (%)	27.7 ± 13.4	26.7 ± 12.2	24.2 ± 19.6
TBM thickness (nm)	1202.9 ± 497.7	1338.9 ± 533.3	1073.3 ± 348.4
Inter-infla score	2.0 ± 0.7	2.1 ± 0.7	**1.3 ± 0.8** [b]
Hyalinosis score	1.8 ± 0.6	1.8 ± 0.4	1.8 ± 0.4
A-sclerosis score	0.6 ± 0.5	0.8 ± 0.4	1.0 ± 0.6
Abbreviations: same as Table 1. a, *vs.* the “None” group. b, *vs.* the Isotype group.			

Among these three groups, interstitial fibrosis, TBM thickness, and arterial injury score were similar. The multiple types group, in which three of the six cases were IgG subclass deposited along TBM only, showed less tubular KIM-1 density and interstitial inflammation than the None or Isotype groups (Fig. 3B).

Discussion

In our study, 67.2% of the DN cases showed linear IgG staining by IF, similar to that observed in previous studies [2]. Unlike other immune complex-mediated glomerular nephropathy, such as membranous nephropathy, DN with positive IgG had negative or trace complements along with GBM or TBM, and electron microscopy showed negative dense deposits along GBM/TBM. Eluates from DN kidneys do not contain anti-GBM antibody [15]. Furthermore, not only IgG but also albumin showed linear expression along GBM in DN cases, suggesting this kind of IgG deposition might occur more frequently as a consequence of renal injury [3, 4, 16].

Not all DN cases had positive expression of IgG subclass, and they showed different positive statuses among DN cases. IgG subclass is a very useful diagnostic tool for several renal diseases, including MN, heavy and light chain deposition disease, proliferative glomerulonephritis with polyclonal IgG deposition, and so on [7]. In our study, we found that IgG1 and IgG2 were major subclasses of GBM IgG expression in DN, which was consistent with another recent paper; however, researchers found no relation between the IgG subclass distribution along the GBM and clinicopathological data [17]. We also found that IgG1 was expressed along both GBM and TBM, while IgG2 was more restrictive along GBM, and IgG3 was more restrictive along TBM. The different locations of IgG subclass deposition are determined by both immunoglobulin profiles and the local environment. While the molecular size is similar among four

subclasses of IgG, the cationic charge and serum concentration decreased in the order of IgG1, IgG2, IgG3, and IgG4 [6, 9, 18]. Owing to the anionic charge feature of GBM, the affinity of IgG subclass to GBM should be higher for IgG1 and IgG2 than IgG4 [19, 20]. Podocyte injury during the early stage of DN recognized the GBM matrix and induced GBM thickness [21]. The glomerular clearance of IgG4 increased, while IgG1 clearance decreased reciprocally in diabetes compared to that in the control [22]. Whether these changes of foamy GBM matrix contribute to all the different IgG subclass attractions remains unclear. For the TBM deposit of IgG, two possible mechanisms may be proposed. First, the glomeruli-filtered IgG is reabsorbed by tubular epithelial cells (TECs), and finally deposited along the TBM. TEC induces endocytosis of IgG via megalin/cubulin complex, and induces transcytosis via neonatal Fc receptor (FcRn) for IgG [23]. In DN, megalin/cubulin expression is increased at early stage and decreased at late stage [24, 25]. One study showed that tubular reabsorption of IgG1 was moderately reduced and that of IgG4 remained at the same level in diabetes compared to that in controls [22]. TBM thickness is also detected in DN. Whether this kind of change contributes to the IgG deposit along TBM is unknown. We found that IgG1 and IgG3 were major subclasses for TBM deposition, potentially arising due to tubular selective reabsorption. Second, the interstitial IgG that leaks from peritubular capillary is trapped by TBM. We found that the group with IgG subclass deposition along TBM-only had a higher level of arteriosclerosis, suggesting this possibility. However, in contrast, no megalin/cubulin expression on the TBM side suggests a lower possibility of this mechanism.

Although IgG deposit is considered as a consequence rather than the cause of DN injury, several studies have indicated that IgG positivity is associated with poorer renal prognosis in DN [2, 17]. In our study, cases with both GBM and TBM IgG subclass deposition showed more proteinuria than those with TBM-only deposition, suggesting severe glomerular injury. However, the TBM-only group showed the lowest serum albumin levels, but not the highest proteinuria levels, suggesting well preserved tubular reabsorption, which was verified by less tubular KIM-1 expression. IgG subclasses are also critical for diagnosis and may correlate with progression, such as in MN [7]. In our study, IgG1 in glomeruli was associated with CD34 loss, thus indicating increased glomerular epithelial cell injury, while IgG2 in glomeruli was associated with GBM thickness, which might be due to IgG2 being the most stable antibody in terms of structure and long-term retention [26]. However, GBM thickening in DN is thought to be the result of podocyte injury, which disrupts the balance of GBM matrix synthesis and degradation [21]. Thickened GBM accompanied by glomerular endothelial cell injury prefers to trap IgG1 and IgG2, which is supported by complete overlap staining of IgG and its subclasses with collagen IV α5 [27]. Furthermore, cases with multiple IgG subclass deposition along TBM had less tubular injury, interstitial inflammation, as well as similar TBM thickness and IFTA score than those with isotype IgG subclass deposition, potentially indicating well preserved and less selective reabsorption by tubular epithelial cells in early-stage DN [22].

The limitations of this study should be noted. First, this is a descriptive study with an interesting finding, but mechanistic studies need to be performed in the future. Second, the sample size of the IgG3 positive group was limited, that resulted in difficulty of analysis of the clinicopathological features of this group, and which negatively impacted the comparison with other groups. Lastly, serum level and urine secretion

of IgG subclass could not be analyzed due to the retrospective nature of the present study, and further prospective research is warranted.

In summary, IgG1 and IgG2 were major IgG subclasses deposited along GBM, while IgG1 and IgG3 were major IgG subclasses deposited along TBM in DN, which were determined by their profiles and severity of glomerular/tubular injury. IgG and its subclass deposition are not causal, but the consequence of renal injury and the positive statuses are associated with different DN injuries.

Abbreviations

DN: Diabetic nephropathy; GBM: Glomerular basement membrane; TBM: Tubular basement membrane; MN: Membranous nephropathy; IF: Immunofluorescence; Scr: Serum creatinine; eGFR: Estimated glomerular filtration rate; LM: Light microscopy; IHC: Immunohistochemistry ; EM: Electron microscopy; IFTA: Interstitial fibrosis and tubular atrophy; TECs: tubular epithelial cells; FcRn: Neonatal Fc receptor

Declarations

Consent for publication

Written informed consent for publication was obtained from each participant.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

XLT contributed to the study design, fund support and paper draft; XJ and FW contributed to the fund and data analyses; XHL and RCY performed the pathological analyses and technical support; YYL and BZ provided supervision; and HCY provided instruction and mentorship. All authors reviewed the manuscript and approved the final version.

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Figures

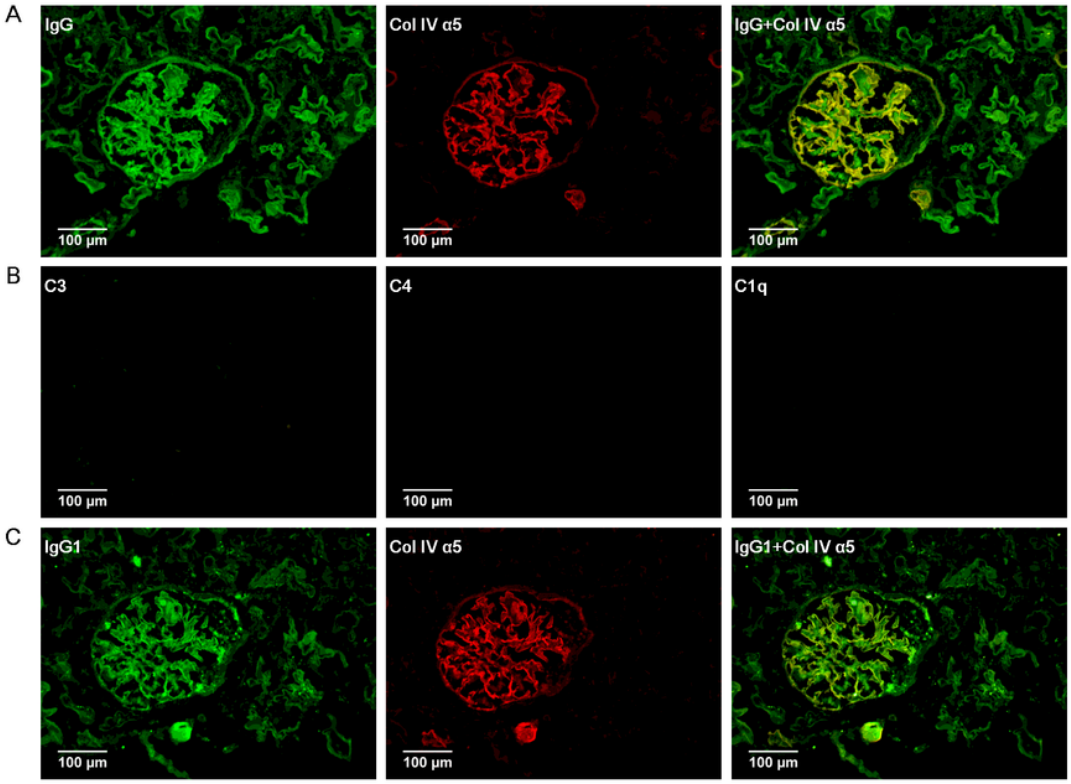

Figure 1

Linear deposition of IgG and IgG1 in diabetic nephropathy. A) Glomerular linear IgG expression (Green), collagen IV α5 expression (Red), and colocalization of IgG and collagen IV α5 in glomeruli (x200) IF. B) Negative C3, C4, and C1q in the same biopsy of figure A (x200) IF. C) Glomerular linear IgG1 expression (Green), collagen IV α5 expression (Red), and colocalization of IgG1 and collagen IV α5 in glomeruli (x200) IF.

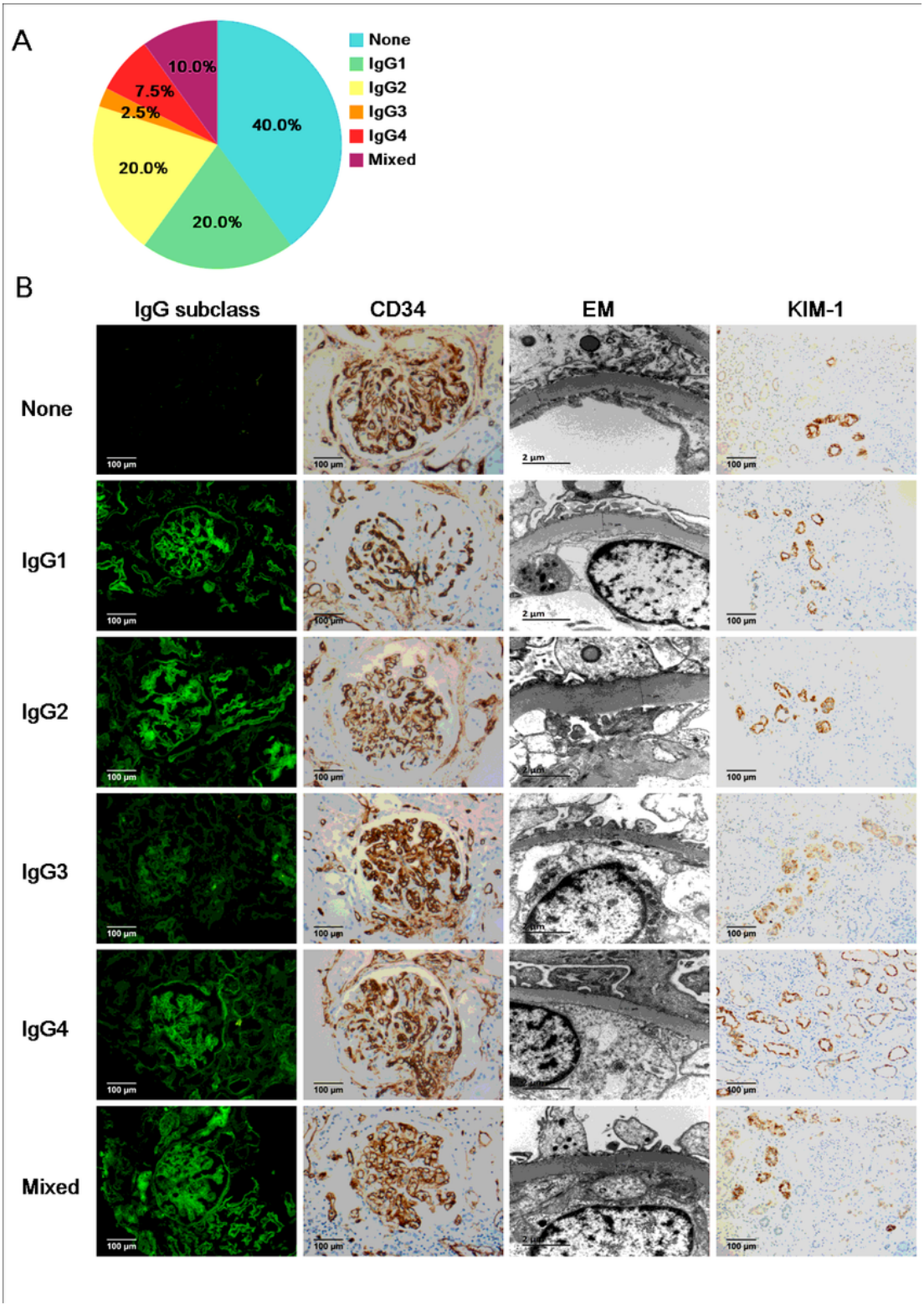

Figure 2

IgG subclass deposition along GBM and its association with pathological features. A) Subgroups of cases with IgG subclass staining along GBM. B) Glomerular IgG subclass staining and associated pathological features. (IgG subclass: x200, IF; CD34: x400, IHC; EM, x10000; KIM-1: x200, IHC)

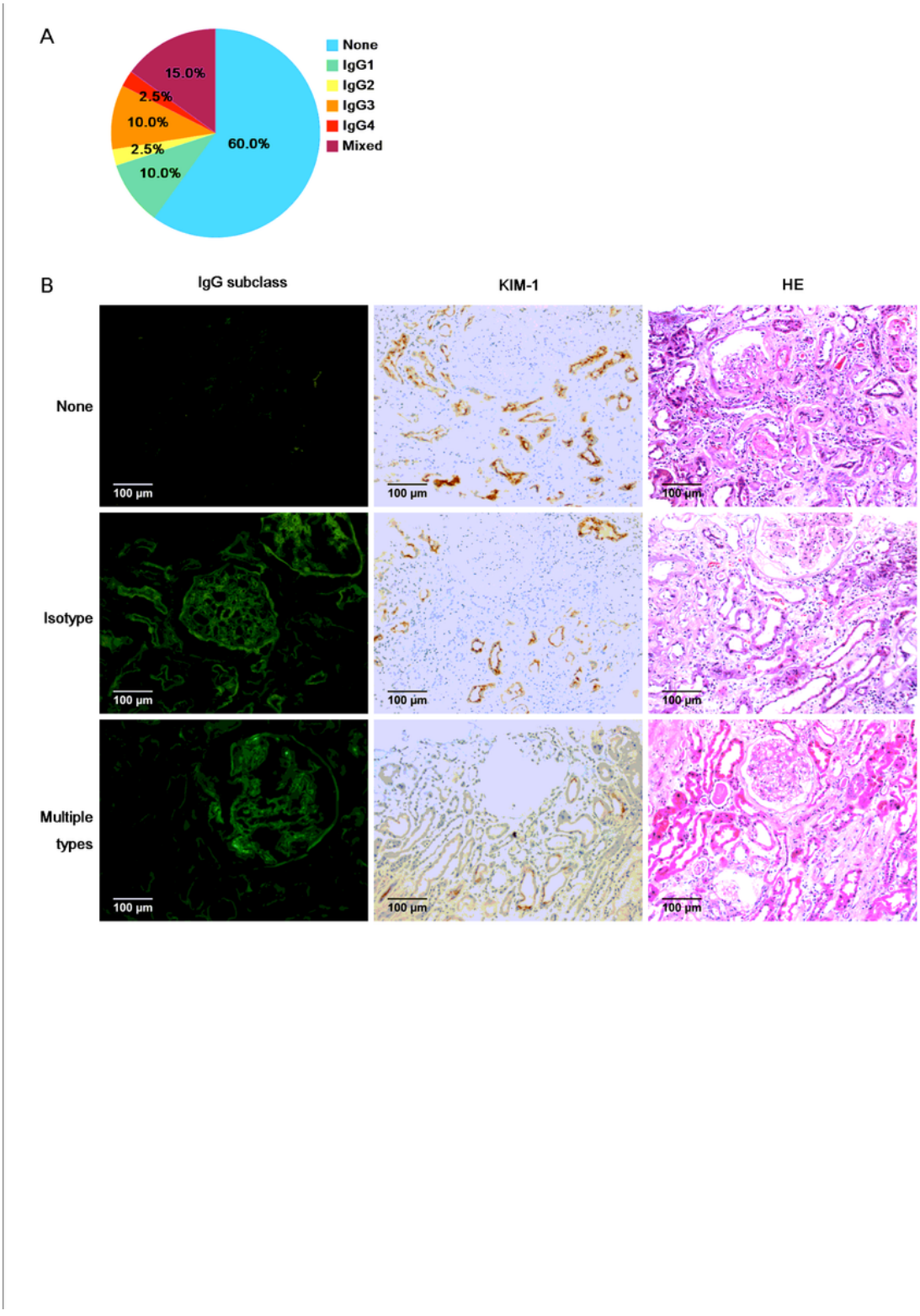

Figure 3

IgG subclass deposition along TBM and its association with pathological features. A) Subgroups of cases with IgG subclass staining along TBM. B) TBM IgG subclass staining and associated pathological features. (IgG subclass: x200, IHC; KIM-1: x200, IHC; HE: x200)