Apolipoprotein E polymorphism and expression in type 2 diabetic patients with nephropathy: clinicopathological correlation

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Abstract

Background. Diabetic nephropathy represents a heterogeneous group of renal pathologies that may be associated with genetic susceptibility. There have been clinical reports on the risk association of diabetic nephropathy with an apolipoprotein E (ApoE) exon 4 polymorphism although its correlations with renal histopathological changes have not been explored.

Methods. A total of 213 adult autopsies with type 2 diabetes and 111 non-diabetic control cases were analysed. Genomic DNA samples were obtained from spleen tissues. The ApoE genotype was determined by PCR-LDR analysis. Histopathological examination of kidney sections was performed in a subset of 51 diabetic and 111 control cases. ApoE protein expression in diabetic carriers with similar clinical status was examined by immunohistochemical staining.

Results. In type 2 diabetes, ε2 carriers (P = 0.04; odds ratio = 5.42; 95% CI: 1.10–26.8) and ε3/ε4 (P = 0.04; odds ratio = 22.5; 95% CI: 1.11–454.90) genotype carriers were more likely to have glomerular hypertrophy than were ε3/ε3 carriers. The ε2 carriers showed an increase in glomerular ApoE protein expression. A correlation between ApoE genotype and nodular glomerulosclerosis was not found.

Conclusions. Our findings confirm the risk association of the ApoE polymorphism with diabetic nephropathy in clinical studies and is the first study demonstrating the correlations between ApoE genotypes, protein expression and structural changes in diabetic nephropathy.

Keywords: apolipoprotein E; diabetic nephropathy; glomerulopathy

Introduction

Diabetic nephropathy is the most common cause of end-stage renal disease (ESRD) in the USA and Europe [1]. With better interventional therapies for cardiovascular complications, type 2 diabetic patients are more likely to survive long enough to develop ESRD. In support of this notion, in western countries, increasing incidence of diabetes-related ESRD has coincided with overall reductions in death rates from heart disease and stroke [2]. Compared to Caucasians, Asians, including Chinese type 2 diabetic patients, have a higher incidence of nephropathy, with 20% having clinical proteinuria and 40% microalbuminuria [3].

Diabetic nephropathy is characterized by clinical albuminuria [4] although there is marked heterogeneity in renal pathologies [5,6]. Multiple causes have been implicated in diabetic nephropathy including hyperglycaemia, hypertension, smoking and dyslipidaemia [7]. In addition, a number of candidate genes have been associated with the initiation and progression of diabetic nephropathy [8,9], including apolipoprotein E (ApoE), aldose reductase and apoC3 [10–12]. An ApoE exon 4 polymorphism is defined by three common variants in the ApoE gene, ε2, ε3 and ε4, respectively, and coding proteins E2 (Arg158 → Cys), E3 (parent isoform) and E4 (Arg158 → Cys) [11]. Several studies have shown that the ε2 allele is associated with an increased risk of type 2 diabetic nephropathy [13,14], macroalbuminuria [15] and renal failure [16], indicating that the ε2 allele increases the risk of renal disease in type 2 diabetes [11]. Despite these clinical studies, the correlations between ApoE genotypes and renal histopathological changes in type 2 diabetes have not been examined. In the present analysis, we examined these associations between genotypes, protein expression and structural changes using autopsy specimens in a case control study.

Subjects and Methods

Autopsy materials

Renal tissues of consecutive autopsy cases were used in the present study to overcome the selection bias of renal biopsy, which is not routinely performed in the management of diabetic nephropathy. Moreover, since not all deaths at major tertiary hospitals are autopsied, we sought to minimize the deliberate bias by including consecutive autopsy cases [10].

Consecutive autopsy cases in the Prince of Wales Hospital were included if they had (1) clinical data on hypertension, plasma glucose or diabetic status [10] and (2) a full autopsy report of renal changes and
causes of death. Figure 1 shows the workflow of this study. Specimens of kidney and spleen were taken at post-mortem examination, fixed in 10% buffered formalin and embedded in paraffin blocks.

**Clinical assessments**

Patients’ hospital records including autopsy reports were reviewed. Clinical data such as blood pressure, plasma glucose, glycosylated haemoglobin (HbA1c) and renal function were assessed 1 month before death to correctly reflect the previous long-term condition of the patient. At the Prince of Wales Hospital, plasma creatinine was measured using the Jaffe method on a Dimension AR system (Dade Behring, Deerfield, IL, USA). Glomerular filtration function was estimated using the formula proposed by the Modification of Diet in Renal Disease (MDRD) Study Group [17]. Hypertension was defined by a blood pressure of 140/90 mmHg or greater or treatment with antihypertensive medications.

Diabetes was defined by the following criteria [18]: (1) treatment with oral anti-diabetic agents and/or insulin therapy, (2) a fasting plasma glucose ≥126 mg/dL (7.0 mmol/L) or (3) a random plasma glucose ≥200 mg/dL (11.1 mmol/L). Exclusion criteria for the diagnosis of type 2 diabetes were (1) diabetes secondary to known causes such as chronic pancreatitis and Cushing’s syndrome and (2) type 1 diabetes defined by presentation with ketoacidosis or requirement of insulin therapy from the disease onset [18]. Using these criteria, we selected 328 type 2 diabetic patients. Among them, 213 type 2 diabetic patients had ApoE genotyping data and thus were included for analysis. All the diabetic cases were treated with oral anti-diabetic drugs or insulin. In addition, 111 age- and sex-matched non-diabetic adult autopsy cases were used as controls. Among the controls, two cases showed known kidney disease before death. One had end-stage kidney disease, and the other subject suffered from renal cell carcinoma.

**Histopathological examination of the kidney**

The renal endpoints of diabetic nephropathy were based on histopathological changes as indicated in the autopsy reports (Figure 2) [19]: (1) diabetic glomerulopathy including glomerular hypertrophy (Figure 2b), diffuse glomerulosclerosis (Figure 2c) nodular lesion (Figure 2d); or (2) glomerular hyaline arteriolosclerosis. Glomerular hypertrophy without co-existing glomerulosclerosis was not diagnostic for diabetic nephropathy. Renal death was defined by clinicopathological diagnosis when renal disease was the principal cause of death.

Figure 2 shows types of renal glomerular lesions that included glomerular hypertrophy, diffuse glomerulosclerosis (DGS), nodular glomerulosclerosis (NGS) and global glomerulosclerosis (GGS) (Figure 2e). Tubulointerstitial lesions refer to tubular atrophy with basement membrane thickening (Figure 3b), arteriosclerosis (Figure 3c) and interstitial fibrosis (Figure 3d) with chronic inflammation (Figure 3e).

**Fig. 1.** Workflow in this study.

**Fig. 2.** Histopathological spectrum of glomerular changes in type 2 diabetes. (a) Normal glomerulus. (b) Glomerular hypertrophy. (c) Diffuse glomerulosclerosis. (d) Nodular glomerulosclerosis with sclerotic nodules (SN) and cellular nodules (CN). (e) Global glomerulosclerosis (GS) and chronic inflammatory infiltrates. HE stain, magnification ×400.

For quality assurance, histopathological examination of kidney tissue sections (4–6 µm) stained with haematoxylin and eosin (HE) was performed in 51 age-, sex- and clinical status-matched diabetic patients and 111 control cases. Histopathological changes including glomerular and tubulointerstitial lesions were assessed. For each case, 20 fields of kidney section were randomly selected at an objective magnification of ×20.
DNA extraction
To improve the DNA quality in archived paraffin blocks, we used the white blood cell-concentrated spleen tissues and a modified DNA extraction protocol [20]. Tissue sections of 4–6 μm were cut from formalin-fixed, paraffin-embedded spleen blocks and collected into an autoclaved microtube (1.5 mL). Genomic DNA was extracted as previously described [20]. After incubation with an 800 μL lysis buffer (protease K 20 mg/mL, 1 M Tris–HCl solution 10 μL, 0.5 M EDTA 2 μL) at 65°C overnight, 700 μL freshly prepared phenol:chloroform (1:1) were added to the supernatant, then this step was repeated, followed by further extraction and purification using chloroform:isoamyl alcohol (24:1). The upper aqueous supernatant was pipetted to a fresh microtube, mixed with 0.1 volume 3 M sodium acetate (pH 5.2) and 2.5 volume 100% ethanol by vortexing and incubated at −20°C for at least 30 min. The precipitated DNA was collected by centrifugation at 9500 g at 4°C for 20 min. The extracted DNA was dissolved in a Tris–HCl EDTA buffer (pH 8.0) after drying it completely at room temperature. DNA quality was examined by gel electrophoresis and assessed by the ratio of absorbance at 260–280 nm. A 260/280 ratio of 1.7 to 1.9 indicates highly pure genomic DNA samples.

Apolipoprotein E genotyping
ApoE genotyping was determined by PCR and ligase detection reaction (LDR). A pair of primers (P1: 5′ TCGGGGCCCCGCTGTGACA 3′, P2: 5′ CACTGATCCGCTGGCGG 3′) was used, with denaturation at 95°C for 30 s, annealing at 68°C for 1 min and extension at 72°C for 1 min for 40 cycles. LDR [21] was performed with two groups of probes (1) P1: 5′ P-CAGTCTCTCCATGCTGGCGGCTTTTTTTTTTTTTTTTTT FAM 3′, P2: 5′ TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTCTGCACCAGGCG GCGCG 3′, and P3: 5′ TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTGG CACCGGCGCGCGCA 3′ for rs429358; (2) 5′ P-CTTCTGCGCG 3′, 5′ TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTGTCCTGTACGT CGCCGCG 3′ and P3: 5′ TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTGG CACCGGCGCGCGCA 3′ for rs7412.). PCR product, ligase and probe mixture reacted in 35 cycles of amplification consisting of denaturation at 94°C for 30 s and annealing and extension at 60°C for 2 min. The LDR product was then sequenced (ABI PRISM 3100 DNA Sequencer). The frequencies of ApoE alleles (ε2, ε3, ε4) and ApoE carriers (ε2 carrier: ε2/ε2) and (ε2/ε3; ε3 carrier: ε3/ε3; ε4 carrier: ε3/ε4, ε4/ε4) [22] were correlated with clinical renal endpoints, pathological lesions and clinical characteristics.

Immunohistochemical staining
In the diabetic group, carriers of ε2/ε3 (N = 9), ε3/ε3 (N = 10) and ε3/ε4 (N = 9) genotypes with matched age, sex and serum creatinine were selected for examination of renal ApoE protein expression using immunohistochemical staining. The primary antibody was the mouse anti-ApoE monoclonal antibody (1:100 dilution, Abcam, Cambridge, MA, USA). Briefly, kidney sections (4 μm) were incubated with the ApoE antibody overnight at 4°C, followed by incubation with a horseradish peroxidase enzyme-labelled polymer for 30 min at room temperature. After colouration by addition of diaminobenzidine chromogen, tissues were counterstained with haematoxylin. The stained slides were examined using a Zeiss Axiosplan 2 imaging microscope (Carl Zeiss Inc., Gottingen, Germany), and representative images were automatically obtained using a digital spot camera (version 3.1 for Windows 95/98/NT; Diagnostic Instruments Inc., Sterling Heights, MI, USA). Representative photos were taken with the same settings: bright field-transmitted light, 24-bit red, green and blue (RGB) per pixel with an exposure time ~2 s. For each case, 10 glomeruli were randomly captured at an objective magnification of ×400 and analysed using a morphometric program (MetaMorph 4.0 image acquisition program for Windows, 1999, Downingtown, PA, USA). The ApoE expression is expressed as the proportion of the ApoE-positive area in glomeruli to the total glomeruli area examined.

Statistical analysis
Observed distributions of genotypes were analysed for deviation from the Hardy–Weinberg equilibrium by chi-square tests with one degree of freedom. Data are expressed as mean ± SD, absolute number or percentage. Means were compared using the Student’s t-test and one-way analysis of variance (ANOVA). Categorical variables were compared using the chi-square test or Fisher’s exact test. The odds ratio (OR) and corresponding 95% confidence interval (CI) were calculated using logistic regression. A
Results

Clinicopathological characteristics

The clinicopathological characteristics of the 213 diabetic patients and 111 control cases are summarized in Table 1. Frequencies of diabetic nephropathy and renal death were higher in the diabetic group than in the control group. Glomerular, vascular and interstitial lesions were more severe in diabetic subjects, but tubular lesions and interstitial fibrosis were similar between the two groups.

Patients with nephropathy had lower calculated glomerular filtration rate and higher HbA1c, plasma creatinine and urea than those without. Other clinical and biochemical characteristics were similar (Table 2). Patients with diabetic nephropathy were more likely to have hypertension, nodular glomerulosclerosis and hyaline arteriolsclerosis (Table 2).

Apolipoprotein E polymorphism and renal histopathology

There was an increased frequency of ε2 allele in the diabetic group compared to the normal group (P = 0.007) (Table 3). Compared to ε3 carriers, ε2 allele carriers were more likely to have renal glomerular hypertrophy (Table 4). On logistic regression analysis, the odds ratio of ε2 carriers was 4.33 (95% CI 1.15–16.26, P = 0.04) (Table 5). After adjustment for potential risk factors (age, sex, hypertension and renal death), the adjusted odds ratio of ε2 and ε4 carriers were 5.42 (95% CI = 1.10–26.80, P = 0.0382) and 22.50 (95% CI = 1.11–454.90, P = 0.0424) (Table 5).

Discussion

This is the first study demonstrating correlation between ApoE genetic polymorphism and histopathological changes of diabetic nephropathy. We found that the ε2 allele was associated with increased severity of glomerulopathy in the
Table 3. ApoE allele and genotype frequencies in type 2 diabetic cases and controls

<table>
<thead>
<tr>
<th>Allele</th>
<th>Type 2 diabetes</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>ε2</td>
<td>19.9 (55)</td>
<td>16.8 (8)</td>
</tr>
<tr>
<td>ε3</td>
<td>79.8 (338)</td>
<td>88.3 (196)</td>
</tr>
<tr>
<td>ε4</td>
<td>7.8 (33)</td>
<td>5.4 (12)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Type 2 diabetes</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>ε2/ε2</td>
<td>1.2 (8)</td>
<td>0.3 (1)</td>
</tr>
<tr>
<td>ε2/ε3</td>
<td>15.2 (32)</td>
<td>9.9 (11)</td>
</tr>
<tr>
<td>ε2/ε4</td>
<td>4.5 (7)</td>
<td>0.9 (1)</td>
</tr>
<tr>
<td>ε3/ε3</td>
<td>66.9 (141)</td>
<td>79.3 (88)</td>
</tr>
<tr>
<td>ε3/ε4</td>
<td>11.3 (24)</td>
<td>8.1 (9)</td>
</tr>
<tr>
<td>ε4/ε4</td>
<td>0.5 (1)</td>
<td>0.9 (1)</td>
</tr>
</tbody>
</table>

Data are % (n).

*P = 0.007 versus ε3 allele group, calculated by the chi-square test.

Table 4. Clinicopathological characteristics of ApoE allele carriers in type 2 diabetes

<table>
<thead>
<tr>
<th>Allele</th>
<th>N = 40</th>
<th>N = 141</th>
<th>N = 25</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (year)</td>
<td>68.9 ± 11.6</td>
<td>70.4 ± 11.1</td>
<td>72.1 ± 10.5</td>
</tr>
<tr>
<td>Male</td>
<td>62.5 (25)</td>
<td>44.0 (62)</td>
<td>36.0 (9)</td>
</tr>
<tr>
<td>Hypertension</td>
<td>57.5 (23)</td>
<td>64.7 (90)</td>
<td>60.0 (15)</td>
</tr>
<tr>
<td>Diabetic nephropathy</td>
<td>73.7 (28)</td>
<td>77.4 (106)</td>
<td>91.3 (21)</td>
</tr>
<tr>
<td>Death due to renal causes</td>
<td>5.1 (2)</td>
<td>6.5 (9)</td>
<td>4.0 (1)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Glomerular lesion</th>
<th>N = 14</th>
<th>N = 34</th>
<th>N = 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>GGS</td>
<td>71.4 (10)</td>
<td>76.5 (26)</td>
<td>66.7 (2)</td>
</tr>
<tr>
<td>NGS</td>
<td>64.3 (9)</td>
<td>76.5 (26)</td>
<td>66.7 (2)</td>
</tr>
<tr>
<td>DGS</td>
<td>21.4 (3)</td>
<td>32.4 (11)</td>
<td>33.3 (1)</td>
</tr>
<tr>
<td>Glomerular hypertrophy</td>
<td>57.1 (8)*</td>
<td>23.5 (8)</td>
<td>66.7 (2)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Tubulointerstitial lesion</th>
<th>N = 70</th>
<th>N = 26</th>
<th>N = 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hyaline arteriolar scleroderma</td>
<td>50.0 (7)</td>
<td>58.8 (20)</td>
<td>100.0 (3)</td>
</tr>
<tr>
<td>Arteriosclerosis</td>
<td>78.6 (11)</td>
<td>79.4 (27)</td>
<td>100.0 (3)</td>
</tr>
<tr>
<td>Tubular lesion</td>
<td>35.7 (3)</td>
<td>32.4 (11)</td>
<td>66.7 (2)</td>
</tr>
<tr>
<td>Interstitial fibrosis</td>
<td>50.0 (7)</td>
<td>50.0 (17)</td>
<td>66.7 (2)</td>
</tr>
<tr>
<td>Normal structure</td>
<td>7.1 (1)</td>
<td>0.0 (0)</td>
<td>0.0 (0)</td>
</tr>
</tbody>
</table>

*P = 0.0421 versus ε3 carrier group, power = 0.6354.

GGS: global glomerulosclerosis; NGS, nodular glomerulosclerosis; DGS, diffuse glomerulosclerosis.

Data are mean ± SD or % (n). They were compared by one-way ANOVA, the chi-square test and Fisher’s exact test.

A form of glomerular hypertrophy and this was coincident with increased glomerular expression of ApoE in the nodular lesions.

Epidemiological studies have reported the association of ApoE ε2 with the onset and progression of renal disease in type 2 diabetic patients [13,23]. In support of these findings, our findings of association of ApoE ε2 polymorphisms with nephropathy accompanied by increased protein expression and structural changes strongly supports a causal relationship. However, the nature of cellular mechanisms underlying the association between ApoE ε2 and diabetic glomerulopathy remains elusive. As one of the major components of lipoproteins, ApoE plays a pivotal role in the metabolism of lipoproteins and lipid remnants through its

Table 5. Associations of ApoE alleles and genotypes with renal glomerular hypertrophy

<table>
<thead>
<tr>
<th>Allele</th>
<th>Crude OR (95% CI)</th>
<th>P</th>
<th>Adjusteda OR (95% CI)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>ε2 carrier (ε2/ε2, ε2/ε3)</td>
<td>4.33 (1.15–16.26)</td>
<td>0.0421</td>
<td>5.42 (1.10–26.80)</td>
<td>0.0382</td>
</tr>
<tr>
<td>ε3 carrier (ε3/ε3)</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>ε4 carrier (ε3/ε4)</td>
<td>6.5 (0.52–81.42)</td>
<td>0.1718</td>
<td>22.50 (1.11–454.90)</td>
<td>0.0424</td>
</tr>
<tr>
<td>Non-ε3/ε3 carrier</td>
<td>4.64 (1.33–16.19)</td>
<td>0.0160</td>
<td>6.72 (1.58–28.59)</td>
<td>0.0100</td>
</tr>
</tbody>
</table>

aAdjusted by age, sex, hypertension and renal death; OR: odds ratio.
ability to bind specific receptors [24]. The genetic polymorphism of ApoE ε2 isoform results in a change from Arg to Cys in residue 158 to form the ApoE ε2 isoform with altered ability to bind its receptors. In this regard, the binding ability of ApoE ε2 to LDL is 50–100 times weaker than that of ε3 or ε4 [25]. The ApoE ε2 isoform is also less competent than E3 and E4 in binding heparin, which is important in the liver for remnant lipoprotein metabolism. On the other hand, ApoE ε3 and ε2 isoforms tend to bind to HDL, whereas ApoE ε4 prefers to bind to VLDL [26]. These changes in the binding capacities of various isoforms of ApoE will give rise to a pattern of increased triglyceride and remnant-like lipoprotein particles in ApoE ε2 carriers, while ApoE ε4 carriers will have the opposite lipoprotein levels [13]. Thus, elevated TG-rich lipoproteins levels in ApoE ε2 carriers may enhance glycosylated or oxidized LDL accumulation in mesangial cells [13] and macrophages [27,28]. The latter can stimulate secretion of cytokines such as interleukin-6, platelet-derived growth factor, intercellular adhesion molecule-1, transforming growth factor-β, monocyte colony-stimulating factor and matrix metalloproteinase-3 [28–31]. All these cytokines have been shown to enhance the production of extracellular matrix proteins, causing expansion of the mesangium. Furthermore, the recruited macrophages may oxidize more LDL particles that intensify renal damage [28]. Similar cytokine reactions and reactive oxygen species are also observed in tubulointerstitialitis [28]. Apart from being a lipid transport protein, ApoE also regulates immunological reaction [32] and promotes cell regeneration after tissue injuries [33]. Due to its limited reduced ability to bind heparin, ApoE ε2 isoform may be less capable than ε3 isoform to inhibit glomerular mesangial cell proliferation [26]. The roles of ApoE isoforms in diabetic nephropathy remain a subject of debate [34–36], although most studies suggest that ApoE ε2 variant increases and ε4 decreases the risk of renal disease in type 2 diabetes [11]. In keeping with our clinical studies, our findings of the genetic–pathological correlations help clarify some of these controversial issues [12]. Compared with the ε3 allele, the ε2 allele was associated with glomerular damage reflected by impaired filtration capacity of glomerular membrane, and extracellular matrix expansion [37]. In the present study, the ε2/ε3 genotype was associated with increased ApoE protein deposition in the mesangial area and nodular lesions, suggesting allele-specific ApoE protein expression in the development of diabetic glomerulosclerosis [38,39]. After adjusting for confounding variables, we also found associations between ApoE ε4 and glomerular hypertrophy in this study, but the protein expression pattern in the ε3/ε4 genotype was different. The ApoE ε4 allele has been reported to be a risk allele for atherosclerosis [40], cerebrovascular disease [41] and Alzheimer’s disease [42]. The alteration in residue 112 of the ApoE ε4 isoform can result in different protein–protein interactions [43] which may account for the reported effects of the ApoE ε4 isoform on activation of the renin–angiotensin system [44], endothelial dysfunction, recruitment and adhesion of macrophages [45], oxidative stress [46] and compromised nerve protection [33]. Thus, it is plausible that ApoE ε2 may directly contribute to glomerular damages while ApoE ε4 may preferentially cause vascular injuries. Both of these can produce glomerular damage, although the low frequency of ApoE ε4 allele in our cohorts does not permit direct testing of this hypothesis.

The kidney is an integrated organ comprising glomeruli, tubules, arteries and arterioles. In type 2 diabetic patients with microalbuminuria, damage to glomerular, vascular and tubulointerstitial compartments of the kidney may vary in different degrees of severity [47]. Our study suggests that the presence of the ApoE ε2 polymorphism contributes to this heterogeneity by increasing glomerular hypertrophy. Experimental studies indicate that ApoE may be an important regulator in the progression of nephropathy. ApoE knock-out mice exhibit hyperplasia of mesangial cells while ApoE lipoproteins inhibited apoptosis induced by oxidized low-density lipoprotein. In vitro studies showed that ApoE stimulated mesangial cell proliferation at 24 h of culture, but inhibited cellular proliferation after 48 h [37].

There are several limitations to this study. Due to generally decreasing autopsy rate, it is difficult to recruit an autopsy cohort of a large sample size. We only repeated the examination of the kidney biopsies in 51 diabetic and 111 control cases. Due to the relatively small sample size, these risk associations may be subject to both type I and type 2 errors. Since not all deaths were autopsied in our hospital, our study is subject to selection bias. However, we have sought to minimize this by using consecutive autopsies.

In summary, there are conflicting data on the risk association between ApoE genetic polymorphisms and diabetic nephropathy in part due to the heterogeneity of study design, definitions and ethnicity. Our findings of the genetic–pathological correlations confirmed the heterogeneity of histopathological changes in type 2 diabetes. In support of our clinical studies [12], the risk association between ApoE ε2 polymorphism and glomerular hypertrophy accompanied by increased protein expression strongly suggests an important role of the ApoE exon 4 polymorphism in the development of diabetic glomerulopathy.

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Conflict of interest statement. None declared.

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Apolipoprotein E in diabetic kidney disease


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