Diabetic Nephropathy: Review

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The Prevalence and Management of Diabetic Nephropathy in Asia

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Abstract

Background: Diabetic nephropathy (DN), especially type 2 diabetes, is now increasing rapidly worldwide, also in Asian countries, and is one of the major long-term vascular complications. The pathogenesis of DN involves both genetic and environmental factors. Around 30–40% of type 2 diabetic patients develop DN despite strict blood glucose and/or blood pressure control. Although it is considered that the genetic background may influence the initiation and progression of DN, the candidate genes are still obscure. Summary: To search for genes that are involved in the susceptibility of DN, a candidate gene approach was taken in the beginning before the development of genome-wide association studies. Although a candidate gene approach can detect rare genetic variants, in advance we need known or presumed pathophysiological knowledge of the specific gene. Investigations using spontaneous animal models are important to determine the pathogenesis and treatment of DN patients. There are many spontaneous animal models, such as the NOD and Akita mice for type 1 diabetes and the Ob/Ob, db/db, Tsumura Suzuki Obese Diabetics, and KK-A₁ mice for type 2 diabetes. Furthermore, the toxicity of persistent hyperglycemia, the activation of reactive oxygen species, systemic and/or glomerular hypertension, microinflammation, dyslipidemia, and other factors are considered to play important roles. Diabetic patients with normoalbuminuria and normal renal function showed typical histological patterns of DN. The discovery of a specific and reliable diagnostic and prognostic biomarker other than albuminuria is urgently needed and indispensable. Since large clinical trials of oral hypoglycemic drugs in renal failure are lacking, these recommendations will need to be regularly updated after results of larger randomized trials with longer follow-up durations are available. Key Messages: It is necessary to summarize the basic and clinical features of DN patients in Asia and to use these for the treatment of such patients. Facts from East and West: The prevalence of DN is increasing in Asia and Western countries alike. The deletion (D) allele of the angiotensin-converting enzyme gene is associated with progression to end-stage renal disease in Asian patients with DN, but this association is uncertain in Europeans. An association between DN and polymorphism of the gene coding for acetyl coenzyme A carboxylase β has been reported in Asian and Western populations. Both in Japan and the US, criteria for diagnosis are a 5-year history of diabetes and persistent albuminuria. Renal biopsy should be

Key Words

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Introduction

Diabetic nephropathy (DN), especially type 2 diabetes, is now increasing rapidly worldwide, also in Asian countries, and it is one of the major long-term microvascular complications occurring in nearly 40% of Japanese diabetic patients. In Japan, over the past two decades, the proportion of individuals with DN with new-onset hemodialysis induction dramatically increased along with increased numbers of diabetes [1]. According to the 2013 annual report by the Japanese Society of Dialysis Therapy (JSDT), the total number of dialysis patients was more than 310,000, and diabetes has become the leading cause of end-stage kidney disease (ESKD; almost 44.0%). Around 30–40% of type 2 diabetic patients develop DN despite strict blood glucose and/or blood pressure (BP) control. The pathogenesis of DN involves both genetic and environmental factors [2]. However, the candidate genes related to the initiation and progression of DN are still obscure. Regarding environmental factors, the toxicity of persistent hyperglycemia, the activation of reactive oxygen species, systemic and/or glomerular hypertension, microinflammation, dyslipidemia, and other factors are considered to play important roles.

Genetic Background in Asia

Although many factors such as hyperglycemia, hypertension, dyslipidemia, obesity, smoking and insulin resistance are involved in the development of DN, these factors are insufficient to predict which diabetic patients will develop or progress to DN. Given the situation that not all patients with poor glycemic and BP control will develop renal disease, and also because ethnic variations and familial aggregation of DN have been observed, genetic factors might contribute to the susceptibility of this disease.

To search for genes that are involved in the susceptibility of DN, a candidate gene approach was taken in the beginning, before the development of genome-wide association studies (GWAS). Although a candidate gene approach can detect rare genetic variants, in advance we need known or presumed pathophysiological knowledge of the specific gene. Moreover, note that this approach can have conflicting results. On the other hand, GWAS usually perform analyses of the entire genome without any presuppositions regarding specific functional features of specific genes.

The renin-angiotensin-aldosterone system (RAAS) is considered to play a crucial role in the development and progression of DN. Indeed, RAAS blockers are widely used in the expectation of renoprotection, such as for antiproteinuric effects in real clinical settings. Angiotensin-converting enzyme (ACE) gene polymorphism has been studied intensively among candidate genes so far. The ACE gene 287-bp Alu repetitive sequences in the intron 16 result in a genetic variation which creates insertion (II) and deletion (DD) homozygous genotypes and insertion/deletion (ID) heterozygous genotype. To sum up the published data, the D allele is responsible for high enzymatic activity, not only in sera but also in renal tissues, and contributes to susceptibility to this disease. Previously, we have also determined I/D polymorphism of the ACE gene in a multicenter trial of ethnically homogeneous Japanese patients with type 2 diabetes (n = 748–1,152) [3, 4]. A recent meta-analysis [5] has demonstrated that the D allele or DD genotype is associated with ESKD susceptibility in Asian patients with DN. The association of the D allele with ESKD was not observed in Caucasian patients with DN, although the DD genotype was.

GWAS for DN has been studied intensively in Japanese patients with type 2 diabetes. Maeda and colleagues [6] successfully identified several genes, such as solute carrier family 12 (sodium/chloride) member 3 (SLC12A3), genes involved in engulfment and cell motil-
ity 1 (ELMO1) [7], neurocalcin δ (NCALD) [8], and acetyl coenzyme A carboxylase β (ACACB) [9]. Although the first three loci did not meet the criteria for genome-wide significance, the locus of ACACB did. Importantly, this association was replicated in independent studies involving not only the same ethnicity, Chinese, but also Caucasians. This gene is considered to be involved in the development and progression of DN via an increase of inflammatory cytokines, such as IL-6 [10].

Lin et al. [11] reported the association of ELMO1 gene polymorphism with DN in a Chinese population, supporting its key role as a candidate gene in the susceptibility of DN. Wu et al. [12] reported the impact of 5, 10-methylenetetrahydrofolate synthetase (MTHFS) polymorphism on type 2 DN in the Taiwanese population used by GWAS. Rs6495446 in MTHFS had no significant effect on the risk of DN in Taiwanese patients. Multivariate logistic regression analysis demonstrated that being male, the duration of diabetes, plasma triglyceride level, and glycemic control were factors that predicted the development of DN [12].

New Spontaneous Animal Model

Investigations using spontaneous animal models are important to determine the pathogenesis and treatment of DN patients. There are many spontaneous animal models, such as the NOD mouse and the Akita mouse for type 1 diabetes, and the ob/ob mouse, db/db mouse, Tsumura Suzuki Obese Diabetics (TSOD) mouse, and the KK-AY mouse for type 2 diabetes [13].

Akita Mouse for Type 1 Diabetes

The nonobese, black-hair C57BL/6 mutant mouse that was found in an Akita colony (Akita, Japan) spontaneously develops early age-onset diabetes and is characterized by an autosomal dominant mode of inheritance. In the Akita mice, a diabetic locus named MODY4 was mapped to chromosome 7 in the region distal to D7Mit189 [14]. Akita mice have type 1 diabetes mellitus caused by a spontaneous point mutation in the Ins2 gene, which leads to the misfolding of insulin, resulting in pancreatic β-cell failure, β-cell mass reduction, and overt hyperglycemia as early as 4 weeks of age. Akita mice develop pronounced and sustained hyperglycemia, high levels of albuminuria, and consistent histological changes, suggesting that these mice may be suitable as an experimental model for DN [15]. There are some reports of kidney injury in Akita mice. At 6 months of age, urinary albumin excretion (UAE) and glomerular filtration rate (GFR) were increased and pathological changes such as glomerular hypertrophy and increases in the mesangial matrix were observed [15, 16]. Moreover, Akita mice also develop hypertension and have echocardiographic evidence of heart failure, which are complications commonly associated with diabetes in humans [17].

KK-AY Mouse for Type 2 Diabetes

The KK mouse, one of the type 2 DN mouse models, was established from a Japanese native mouse strain via inbreeding by Kondo et al. in 1957 [18]. Male KK mice are generally considered to be a polygenic disease model and spontaneously exhibit type 2 diabetes associated with hyperglycemia, including high levels of HbA1c, hyperinsulinemia, mild obesity, and microalbuminuria. Since the phenotypic characteristics of KK mice are not especially pronounced, the KK-AY mouse was established by Nishimura in 1969 [19]. This mouse was produced by the transfer of the yellow obese gene (AY allele) into the KK mouse. In 2006, researchers reported that the pathological changes in the glomeruli of KK-AY mice were consistent with those in the early stages of human DN [20]. The urinary albumin/creatinine ratio of KK-AY mice was significantly higher than that of nondiabetic BALB/c mice at all time points (p < 0.001). The levels of body weight in both KK-AY and BALB/c mice increased gradually after 8 weeks of age [20]. However, these levels were significantly higher in KK-AY mice than in BALB/c mice (p < 0.001). The levels of HbA1c were also significantly higher in KK-AY mice than in BALB/c mice (p < 0.001). In periodic acid-Schiff (PAS) and periodic acid-methenamine silver stains of specimens from KK-AY mice, segmental sclerosis was observed in some glomeruli at 20 weeks of age. AGEs and TGF-β protein appeared to be localized in the glomerular mesangial areas. Thus, these products have been implicated in the pathogenesis of human DN. It appears that the KK-AY mouse is considered to be a suitable animal model for type 2 DN [20].
Clinical Classification in Japan

DN is considered to be a major cause of ESKD in the world. Although renal biopsy is not performed on all patients with diabetes and the onset of type 2 diabetes is unclear, clinical diagnostic criteria are usually used in Japan. Among these clinical findings, which are shown in Table 1, the presence of diabetic retinopathy and/or neuropathy is a key parameter for clinical diagnosis. Persistent proteinuria or albuminuria are also important clinical markers. If diabetic patients exhibit severe hematuria and/or cellular casts, i.e. red blood cell casts, it is important to rule out malignancies using urinary cytology, ultrasonography, and computerized tomography (CT) scans, and perform renal biopsies for differential diagnosis. An increase in GFR (Ccr) is observed at the onset of type 2 diabetes. Enlargement of the kidneys detected by ultrasonography or CT scans is also observed in diabetes.

Based on these clinical features, the committees of both the Japan Diabetes Society (JDS) and the Japanese Society of Nephrology (JSN) have revised the classification of DN. The revised classification is adapted to the chronic kidney disease (CKD) classification (CGA classification) of the JSN made in 2012. Patients with DN are classified into five stages as follows: stage I (normoalbuminuria stage), showing normal or increased estimated GFR (eGFR, more than 30 ml/min of eGFR); stage II (microalbuminuric stage), showing microalbuminuria (30–299 mg/g Cr of UAE, more than 30 ml/min of eGFR); stage III (macroalbuminuric stage), showing macroalbuminuria (more than 300 mg/g Cr of UAE, more than 30 ml/min of eGFR), and stage IV (renal failure stage), showing a decline in renal function (less than 30 ml/min of eGFR). In stage IV, patients also show uremic findings such as polyuria, oliguria, nocturia, renal anemia, hypertension, edema, nausea, and vomiting. Stage V is the dialysis stage (Fig. 1).

New Biomarkers

Albuminuria is one of the first asymptomatic clinical manifestations of microvascular injury in diabetes. In Japan, early DN (stage II) is defined by the presence of microalbuminuria in a clinical setting, although we make a differential diagnosis when another disease is suspected. Although albuminuria lacks specificity and sensitivity as a prognostic biomarker for progressive DN, a growing body of evidence suggests that the presence of mild degrees of albuminuria shows an increased risk of cardiovascular disease. Recently, Moriya et al. [21] demonstrated that the renal histological pattern was heterogeneous and related to CKD stages but not to albuminuria categories. Given the situation that certain Japanese diabetic patients with normoalbuminuria and normal renal function showed typical histological patterns of DN, the discovery of a specific and reliable diagnostic and prognostic biomarker, other than albuminuria, is urgently needed and would be indispensable. Rim et al. [22] performed a cross-sectional study to identify and determine the sociodemographic and health-related factors associated with diabetic retinopathy and DN screening in Korea. The relatively low screening rates in Korea compared to the Western countries are likely to be due to the difference in the health system, economic situations and national demographics [22]. Chong et al. [23] reported the role of renal biopsy in diabetic patients with renal involvement. In the DN subgroup, Indian patients had a significantly shorter duration of diabetes on renal biopsy compared with Malays and Chinese patients. For nondiabetic renal disease patients, useful clinical markers include the presence of acute renal failure and microscopic hematuria. They concluded that renal biopsy should be considered in type 2 diabetic patients with nephropathy [23].

Podocytes

Glomerulosclerosis in several human kidney disorders is associated with podocyte injury. The number of podocytes per glomerulus may be a podocyte injury parameter that could provide prognostic information in patients with DN. Progressive DN is associated with a reduction in the number of podocytes per glomerulus. The remaining podocytes are obliged to grow and extend their foot processes to maintain coverage of the same area. Since podocytes do not undergo mitosis in adults, the only way to respond to injury is by cell hypertrophy. Thus, the increased podocyte surface area would reflect the extent of podocyte hypertrophy. Macedo et al. [24] reported that the control of hyperglycemia prevented glomerular basement membrane (GBM) thickening in early and late (12 months) alloxan-induced type 1 DN and also prevented a reduction in the number of podocytes. Ishikawa et al. [25] previously reported that podocyte injury might provide additional prognostic information in diabetic KK-A'y mice. In light microscopy, diffuse expansion of glomerular mesangial matrices was observed in diabetic KK-A'y mice. Sclerosed lobules with several homogenous PAS-positive hyaline caps were observed at 20 weeks of age. The mean podocyte numbers per glomerulus in diabetic KK-A'y mice (mean ± SD, 6.73 ± 1.78) and KK-A'y mice (5.94 ± 2.46) were significantly lower than those in nondiabetic BALB/c mice (8.81 ± 2.37; KK-A'y vs. BALB/c: p < 0.05, KK vs BALB/c: p < 0.01). The mean number of podocytes per 1,000 μm² of glomerular area in diabetic KK-A'y mice and KK mice was also significantly lower than that in non-diabetic BALB/c mice (p < 0.01). The general mechanisms of podocyte loss are thought to occur via the detachment of intact podocytes from the GBM due to necrosis, apoptosis, and/or autophagy. The apoptosis of podocytes has been determined in cell cultures. In this study, TUNEL staining was performed to detect the number of apoptotic cells per glomerulus in KK-A'y mice. An apoptotic cell was observed in only 1 out of 30 glomeruli. However, Gu et al. [26] reported that monocyte chemotactic protein (MCP)-1 was induced by AGEs and N(ε)-(carboxymethyl) lysine (CML) in the differentiated podocytes. The MCP-1/cysteine-cysteine chemokine receptor 2 (CCR2) system is speculated to be involved in the apoptosis of podocytes in DN.

In patients with DN, there is no direct evidence for apoptosis as a major mechanism of podocyte loss. The detachment of intact podocytes from the GBM has been reported in Japanese diabetic patients. Nakamura et al. [27] indicated that the podocytes in urine samples may be a useful marker of disease activity in patients with DN.

Tumor Necrosis Factor Receptors

Tumor necrosis factor (TNF)α binds its two cell surface receptors (TNFRs, TNFR1 and TNFR2) and exerts biological responses. Although the role of TNF and its receptors in kidney disease, especially in DN, is still not clarified, circulating soluble TNFR levels have recently emerged as strong predictors associated with future GFR decline, ESKD, and all-cause mortality in patients with both types of diabetes in the Joslin Kidney Study [28, 29]. Note that this biomarker is very robust after accounting for HbA1c, albuminuria, GFR, and other inflammatory biomarkers such as TNFα, CRP, and IL-6. Moreover, it is interesting to note that both TNFRs are equivalent predictors in spite of their completely different functions.
After this report, replication studies have been performed and positive data have been published. Unfortunately, the availability of this biomarker is still not fully examined in Japanese diabetic patients.

**Mindin**

We performed a microarray analysis using isolated glomeruli from diabetic KK mice for searching for specific genes that are involved in the development and progression of DN. In this analysis, we focused on mindin (also called spondin 2, SPON2) as a possible biomarker for several reasons [30, 31]. Mindin protein expression levels in the glomeruli of KK mice were significantly up-regulated with the progression of DN, they were mainly localized in podocytes by immunohistochemical staining and were a secreted protein. Moreover, previous reports demonstrated that mindin plays a role in immune response and inflammation. Indeed, urinary mindin expression levels are significantly higher in advanced-stage KK mice than in early-stage mice. In addition, the urinary levels of mindin were higher in diabetic patients than in healthy individuals and increased gradually with the progression of DN.

**Drug Therapy**

Zanchi et al. [32] concluded that regular kidney function monitoring with renal impairment and adjustment of antidiabetic drug therapy according to GFR and pharmacokinetic data are of major importance. Since large clinical trials of oral antidiabetic drugs (OADs) in renal failure are lacking, these recommendations will need to be regularly updated after results of larger randomized trials with longer follow-up durations are available [32].

**Incretin-Related Drug: DPP-4 Inhibitor**

Most OADs are contraindicated in advanced kidney disease. The launch of DPP-4 inhibitors opened a new window in the treatment of diabetic kidney disease. Now, DPP-4 inhibitors have become one of the best-selling OADs in Japan because this drug has a small risk for hypoglycemia and weight gain and is available with relative safety. Moreover, a recent meta-analysis has reported that this drug might exhibit a better glucose-lowering efficacy in Asian populations than in other ethnic groups [33]. Differences in body mass index (BMI) across ethnic groups may mediate the HbA1c lowering efficacy of DPP-4 inhibitors. Iwasaki et al. [34] demonstrated that the reduction of HbA1c by DPP-4 inhibitors showed a significant association with the estimated intake of fish in food records and serum levels of EPA and DHA in 72 untreated Japanese patients with type 2 diabetes, suggesting that the efficacy of this drug might be linked to the diet style (Japanese- vs. Western-style food).

A large number of experimental studies using diabetic models demonstrated that DPP-4 inhibitors protect the vascular endothelium and reduce albuminuria through ameliorating oxidative stress and inflammation. In humans, DPP-4 inhibitors partially reduce albuminuria independent of the improvement in glycemic controls [35]. There is not much information about the efficacy of DPP-4 inhibitors, especially in patients with nephropathy, although the MARLINA study (Efficacy, Safety, and Modification of Albuminuria in Type 2 Diabetes Subjects with Renal Disease with Linagliptin) is ongoing.

**RAAS Inhibitors: ACE Inhibitor, Angiotensin Receptor Blockers, and Direct Renin Inhibitor**

Angiotensin II (Ang II) exerts both hemodynamic effects, leading to increased glomerular capillary pressure, and nonhemodynamic effects such as cell proliferation and extracellular matrix accumulation. These effects are mediated through the interaction of Ang II with its angiotensin type 1 (AT1) receptor. ACE inhibitors and angiotensin receptor blockers (ARBs) have been demonstrated to improve glomerular hemodynamics and structure in patients with DN.

In 2002, Katayama et al. [36] reported a double-blind randomized clinical study using two ACE inhibitors, imidapril and captopril, and also a placebo (Japan-IDDM: Japanese trial of ACE inhibitors on renal protection against nephropathy in type 1 diabetes). Imidapril is an ACE inhibitor without sulfhydryl radical and it is a pro-drug of imidaprilat. It has the same potency as an active form of enalapril and two times higher potency than that of captopril in inhibiting ACE activity. A total of 79 eligible cases were randomized to receive 37.5 mg of captopril (n = 26), 5 mg of imidapril (n = 26), or placebos (n = 27) daily in a double-blind manner. UAE, determined every half year, was significantly decreased by the ACE inhibitors (placebo vs. ACE inhibitors, p = 0.001; placebo vs. captopril, p = 0.043; placebo vs. imidapril, p < 0.001) during the study period (mean, 1.48 years). Glycemic and BP control significantly affected UAE. Systolic BP in the placebo group tended to be higher by 7–10 mm Hg throughout the study. Researchers suggested that the
ACE inhibitors, imidapril and captopril, prevent the increase in UAE in micro- and macroalbuninuric patients with type 1 diabetes and that the target BP might be less than 130/80 mm Hg [36].

In 2011, Imai and Chan and colleagues [37] examined the effects of olmesartan, an ARB, on the primary outcome of doubling of serum creatinine, ESKD and death in type 2 diabetic patients with overt nephropathy from Japan and China. A total of 577 patients (377 Japanese, 200 Chinese) treated with antihypertensive therapy and who received concomitant ACE inhibitors (n = 424, 73.5%) were given either olmesartan once daily (n = 288) or a placebo (n = 289) over 3.2 ± 0.6 years (mean ± 1 SD). In the olmesartan group, 116 developed the primary outcome (41.1%) compared with 129 (45.4%) in the placebo group (p = 0.791). Olmesartan significantly decreased BP, proteinuria, and rate of change in the reciprocal serum creatinine. Cardiovascular death was higher in the olmesartan group than in the placebo group. Major adverse cardiovascular events and all-cause deaths were similar between the two groups. Hyperkalemia was more frequent in the olmesartan group than in the placebo group. It is concluded that olmesartan was well tolerated but did not improve the outcome on top of the ACE inhibitor [37]. In patients with DN, combination therapy with an ACE inhibitor (lisinopril) and an ARB (losartan) decreases proteinuria but its safety profile and effects on the progression of kidney disease are still uncertain [38]. There was no benefit with respect to mortality or cardiovascular events. The combination therapy increased the risk of hyperkalemia and acute kidney injury. It is concluded that a combination therapy with an ACE inhibitor and an ARB was associated with an increased risk of adverse events among patients with type 2 DN in the USA [38]. In patients with type 2 diabetes and nephropathy, combination treatment with aliskiren (direct renin inhibitor, DRI) plus losartan may have renoprotective effects that are independent of the BP-lowering effects. Daily treatment with 300 mg aliskiren, as compared to a placebo, reduced the mean UAE by 20% (95% confidence interval, 9–30; p < 0.001), with a reduction of 50% or more in 24.7% of patients who received aliskiren, as compared to 12.5% of those who received the placebo (p < 0.001) [39].

Others

Na et al. [40] reported the effect of beraprost sodium (BPS) on arterial stiffness in patients with type 2 DN. BPS is a prostacyclin analog with vasodilatory and antiplatelet effects. This clinical trial is the first to investigate the effects of BPS on changes in cardiovascular biomarkers, albuminuria, renal function, and lipid profiles in patients with DN.

The study for a randomized, double-blind and placebo-controlled clinical trial of Shenyan Kangfu tablet (SYKFT), a Chinese patent medicine for DN, was performed by Wang et al. [41]. SYKFT is a new formulation of an existing and widely acclaimed Chinese herbal tea. The results of this trial will help to provide evidence-based recommendations for clinicians.

Conclusion

Type 2 DN is now increasing rapidly worldwide, including in Asian countries. Although the pathogenesis of DN involves both genetic and environmental factors, the candidate genes related to the initiation and progression of this disease are still obscure. Regarding environmental factors, the toxicity of persistent hyperglycemia, activation of reactive oxygen species, systemic and/or glomerular hypertension, microinflammation, dyslipidemia, and other factors are considered to play important roles. In this article, we have summarized the basic and clinical features of DN in Asia.

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Disclosure Statement

The authors have no conflicts of interest to declare.

References

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