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Do inflammatory cytokine genes confer susceptibility to diabetic nephropathy?

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Microinflammation has been recognized as an important process for the progression of diabetic nephropathy. Inflammatory cytokines, such as interleukin-6 produced by infiltrating cells or renal cells, play important roles in the pathogenesis of diabetic nephropathy. Although the mechanisms underlying the regulation of these cytokines in the kidneys of patients with diabetes mellitus remain unclear, genetic variations in the genes encoding the inflammatory cytokines might confer susceptibility to the disease by altering their functions or expressions.

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Genetic susceptibility plays an important role in the pathogenesis of diabetic nephropathy. Efforts, including the genome-wide association study (GWAS), have been invested worldwide to identify the susceptibility gene for diabetic nephropathy. GWAS is considered a powerful and promising approach; however, to date, it has not completely covered the human genome. Thus, the candidate gene approach, along with an appropriate analysis, continues to be the method of choice to evaluate the contribution of the genes of interest in conferring susceptibility to the disease. Ng *et al.*[1] (this issue) have now reported the association of the interleukin (IL)-6 gene with reduced glomerular filtration rate in patients with type 2 diabetes; their approach is a good example of evaluating the association of a gene with disease susceptibility. I will discuss whether the inflammatory cytokine gene *IL-6* is a susceptibility gene for diabetic nephropathy.

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Role of inflammatory cytokines in the pathogenesis of diabetic nephropathy

Traditionally, chronic alterations in metabolic and/or hemodynamic factors have been recognized as the main causes of renal injury in patients with diabetes mellitus. In recent years, these conventional mechanisms have been considered only partially responsible for the development and/or progression of the disease. Further, several other factors, such as the activity of reactive oxygen species or various inflammatory processes, have been shown to play important roles in the pathogenesis of diabetic nephropathy; these factors are not completely independent but interact with each other.

For many years, diabetic nephropathy was believed to be a noninflammatory disease, although inflammatory-cell infiltration was observed in the kidneys of some patients with diabetes mellitus. Furuta *et al.* reported a considerable amount of macrophage infiltration in the kidney that was moderately affected with diabetic nephropathy.[2] Another group of researchers observed macrophage infiltration not only in the glomeruli but also in the interstitium of the kidneys of patients with diabetic nephropathy.[3] Most researchers did not focus enough on the increase in the number of inflammatory cells in a diabetic kidney, probably because the degree of infiltration in a diabetic kidney was lower than that observed in proliferative glomerulonephritis. Recently, low-grade or subclinical inflammation, termed microinflammation, has been shown to play an important role in the pathogenesis of atherosclerosis and also in microvascular diseases. In addition, it has been shown that suppression of macrophage infiltration by radiation[4] or by the use of immunosuppressive agents[5] could ameliorate the development and/or progression of diabetic glomerular injury in experimental diabetes mellitus. On the basis of all these observations, we speculate that inflammatory processes contribute to the development and progression of diabetic nephropathy.

The first step of macrophage infiltration is the attachment of mononuclear leukocytes to vascular endothelial cells. This attachment is regulated by adhesion molecules such as intercellular adhesion molecule-1 (ICAM-1), E-selectin, and P-selectin, or by chemokines such as monocyte chemotactic protein-1 (MCP-1). The expressions of ICAM-1, E-selectin, and P-selectin were increased in the kidneys of patients with diabetic nephropathy[3] and in the kidneys of rats with streptozotocin-induced diabetes. Interestingly, Okada *et al.* have demonstrated that ICAM-1 knockout mice were protected from the progression of albuminuria, glomerular hypertrophy, and interstitial fibrosis at 6 months after the induction of diabetes mellitus, whereas no difference was observed in the pathological degree of albuminuria between ICAM-1-null mice and wild-type mice at 1 month after the injection of streptozotocin.[6] These results suggest that the microinflammation observed in the diabetic kidney possibly contributes to the progression of the disease rather than its onset.

Inflammatory cytokines such as IL-1, IL-6, and IL-18, which are produced by the infiltrating macrophages, might contribute to the progression of renal injury either directly or indirectly. Further, increased expression of these cytokines has been reported in the kidneys of patients with

diabetes mellitus[7] or experimental diabetes mellitus.[8] The increase in the systemic and/or tissue expressions of these cytokines was reported to correlate with the severity of diabetic nephropathy or with urinary albumin excretion. These findings support the contribution of microinflammation to the progression of diabetic nephropathy, although precise mechanisms underlying the process of microinflammation and its contribution to the progression of renal injury remain to be elucidated.

Genetics of diabetic nephropathy

On the basis of cumulative evidence, it is suggested that genetic susceptibility plays an important role in the pathogenesis of diabetic nephropathy. Krolewski *et al.* reported that cumulative incidence of diabetic retinopathy increased linearly with increasing duration of diabetes, but the occurrence of nephropathy was extremely rare at 20–25 years after the onset of diabetes, and only a modest number of individuals with diabetes (~30%) developed diabetic nephropathy.[9] Familial clustering of diabetic nephropathy was also reported in both type 1 and type 2 diabetes, strongly suggesting the association of genetic factors with diabetic nephropathy. To date, extensive research has been performed to identify the susceptibility gene for diabetic nephropathy. Several candidate genes, such as those for the renin–angiotensin system, have been shown to be associated with susceptibility to the disease in several independent cohorts. However, most genes responsible for conferring susceptibility to diabetic nephropathy remain unidentified thus far (Figure 1). Recently, GWASs on several common diseases, such as type 2 diabetes, were conducted, and these analyses successfully identified convincing candidate genes for the diseases. With regard to diabetic nephropathy, we genotyped more than 80,000 single-nucleotide polymorphism (SNP) loci and consequently identified solute carrier gene family 12 member 3 (*SLC12A3*),[10] engulfment and cell motility 1 (*ELMO1*),[11] and neurocalcin-δ (*NCALD*)[12] as novel candidates for susceptibility genes for diabetic nephropathy. However, further replication studies are required to validate the effects of these genes.

Association of inflammatory cytokine genes with susceptibility to diabetic nephropathy

As mentioned above, inflammatory cytokine genes might be candidate genes for conferring susceptibility to diabetic nephropathy; several genes, such as *IL-1*, *IL-6*, *IL-18*, and *MCP-1*, have been examined in this regard thus far. Ng *et al.* [1] (this issue) report the association of a haplotype of the *IL-6* gene with reduced renal function in diabetic nephropathy. The *IL-6* gene was found on chromosome 7p21, which contains the locus related to reduced glomerular filtration rate in type 2 diabetes. They examined six representative SNPs in the *IL-6* gene—five tagging SNPs and one previously reported promoter SNP (rs1800796; –634G>C)—and showed that the haplotype comprising these six SNPs was significantly associated with impaired renal function but not with albumin excretion in patients with type 2 diabetes. The approach adopted by Ng *et al.*[1] is superior to analysis of single SNPs; it is considered a good and standard example of candidate gene analysis that completely covers the genes of interest. Their finding is consistent with a previous finding that microinflammation is probably involved in the progression of diabetic nephropathy rather than in the onset of the disease. The association of the *IL-6* gene with diabetic nephropathy has also been examined in two other independent studies. Kitamura *et al.* examined the association of rs1800796 (–634G>C) with albuminuria,[13] and Abrahamian *et al.* examined the association of rs1800795 (–174G>C) with albuminuria.[14] Because the numbers of study subjects in all of these studies, including that of Ng *et al.*,[1] were not large enough to enable the evaluation of the true association, the results were not conclusive. Furthermore, the mechanism by which the above-mentioned haplotype contributes to the susceptibility to impairment of renal function has not been elucidated, although some functional significance of the SNPs has been shown by Fishman *et al.*[15] and Kitamura *et al.*[13] Therefore, further validation of the association of the *IL-6* gene polymorphisms with the disease is required. Several other inflammatory cytokine genes, including the *IL-1* gene cluster on chromosome 2q and tumor necrosis factor-α (*TNF*-α) on chromosome 6p21, and chemokines and their

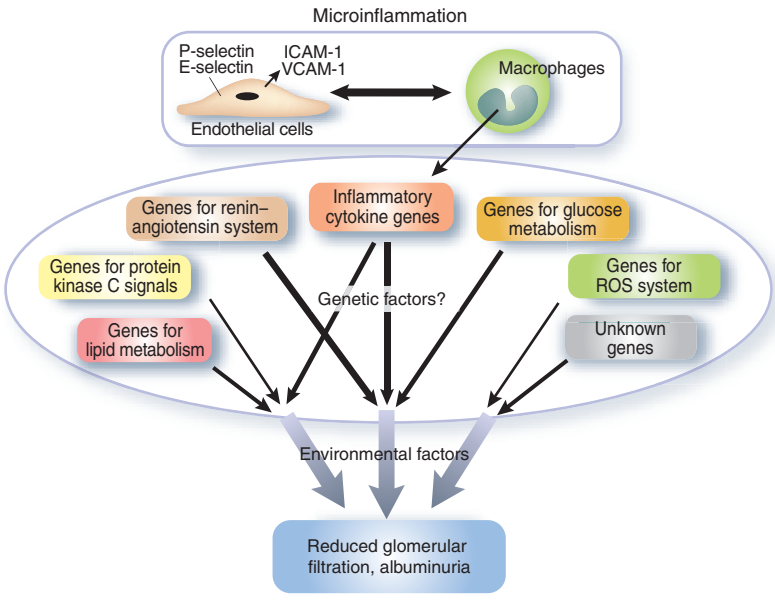

Figure 1 | Microinflammation and diabetic nephropathy. A combination of multiple genetic and/or environmental factors is considered to contribute to the pathogenesis of diabetic nephropathy. Inflammatory cytokine genes, such as *IL-6*, might be good candidates for conferring susceptibility to diabetic nephropathy. ICAM-1, intercellular adhesion molecule-1; VCAM-1, vascular cellular adhesion molecule-1; ROS, reactive oxygen species.

receptors have also been examined thus far. However, even these studies could not draw a definite conclusion.

Recent development in the SNP typing technology and collation of information regarding linkage disequilibrium in the human genome have facilitated GWAS and candidate gene analyses in a large number of subjects by using tagging SNPs that can completely cover the gene or locus of interest. Because, in the case of common genetic variations, the genotype-specific differences in biological functions are expected to be very small, an appropriate association study such as that conducted by Ng *et al.*[1] with a sufficient number of subjects, probably several thousand case and control subjects, should be performed for each candidate gene before evaluation of the functional significance of genetic variations.

Inflammatory cytokine genes might be good candidates for genes that confer susceptibility to diabetic nephropathy. Moreover, well-organized approaches, such as GWASs for diabetic nephropathy, are being conducted in several independent populations; a conclusion regarding the susceptibility genes for diabetic nephropathy may be drawn in the near future.

DISCLOSURE
The author declared no competing interests.

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Gender differences in chronic kidney disease

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Women live longer than men. Can this phenomenon be explained by chronic kidney disease (CKD)? Gender differences in the prevalence and incidence of CKD are discussed.

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Women live longer than men. Can this phenomenon be explained by chronic kidney disease (CKD)? Proteinuria is a known risk factor for cardiovascular disease (CVD) and mortality. Recently, a low estimated glomerular filtration rate (eGFR) *per se* has also become widely accepted as a risk factor for CVD and mortality.[1] The high incidence of infection and malignancies in the elderly population may be, in part, due to CKD. The mortality rate increases with a decline in glomerular filtration rate (GFR) and is highest among patients with end-stage renal disease (ESRD). Therefore, an estimation of GFR is recommended among patients with CVD. In such patients, serum creatinine should be examined to determine the eGFR. The World Health Organization now considers kidney disease a major chronic disease.

CKD is defined as either kidney damage indicated by urine, imaging, and histologic findings, or a low eGFR, less than 60 ml/min/1.73 m^2, for more than 3 months. GFR is calculated by either the Modification of Diet in Renal Disease (MDRD) Study equation or the Cockcroft–Gault formula. The concept of CKD was developed to educate physicians and the general public in the prevention of ESRD and other related medical complications. The mechanisms underlying the increased risk of CVD in CKD patients, however, are not well understood. In addition to the conventional risk factors for CVD, CKD patients often have associated non-conventional risk factors such as volume expansion, sympathetic overactivity, sleep disturbance, hypoxia, increased oxidative stress, dyslipidemia, anemia, and serum calcium and phosphate

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