Genetic factors related to progression of type 2 diabetic nephropathy in Japanese

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Abstract
Presented herein is an examination of the genetic factors related to progression of type 2 diabetic nephropathy in Japanese patients. Epidemiological evidence strongly suggests that genetic susceptibility has a major role in the development of nephropathy in patients with type 2 diabetes. It was found that the DD genotype of angiotensin converting enzyme polymorphism is associated with progression of Japanese type 2 diabetic nephropathy. Genetic analysis of the corresponding chromosomal regions in humans, and examination of candidate genes residing at the loci, may provide a more thorough understanding of genetic factors involved in human type 2 diabetes.

Key words: Angiotensinogen, Disease susceptibility, Genotype, Polymorphism, Proteinuria

INTRODUCTION
Diabetic nephropathy is a major complication of type 2 diabetes mellitus and is the most important cause of end-stage renal failure (ESRF) in Japan. Poor blood glucose and/or blood pressure control may have an important role in the initiation and development of diabetic nephropathy. Diabetic nephropathy is observed in up to 30% of patients who have type 2 diabetes mellitus for more than 10 years (1-3). Over the last decade, it has been shown that diabetic nephropathy clusters in type 2 diabetic families. In Pima Indians, proteinuria was found to occur in 14% of diabetic offspring if neither diabetic parent had proteinuria. The incidence increased to 23% if one diabetic parent had proteinuria, and to 46% if both diabetic parents had proteinuria (4). Familial clustering of ESRF in African-Americans was reported by Freedman et al (5,6). Among patients with type 2 diabetes mellitus and ESRF, 37% reported having a close relative with ESRF. Among age- and sex-matched type 2 diabetic cases without nephropathy, only 7% reported ESRF in a close relative. In 2001, more than 30% of Japanese patients who were newly accepted for regular dialysis had diabetic nephropathy (total number, 12 186) (3). Familial aggregation of diabetic nephropathy has been noted both in Pima Indians (4) and Caucasians (7). Because diabetic nephropathy occurs in familial clusters and not all patients with poor metabolic control develop nephropathy, genetic factors may contribute to susceptibility to this disease. Although many candidate genes in patients with diabetic nephropathy have been analyzed, the candidate genes related to initiation and progression are still obscure in patients with type 2 diabetic nephropathy.

The purpose of this review is to report our work on the genetic factors related to progression of type 2 diabetic nephropathy in Japanese patients.
GENETIC APPROACH TO TYPE 2 DIABETIC NEPHROPATHY

Two different strategies are available in a search for genes responsible for susceptibility to diabetic nephropathy. The first strategy is a genome-wide screening using type 2 diabetic sibling (sib) pairs concordant or discordant for nephropathy. This method can determine the chromosomal location responsible for diabetic nephropathy, but it is difficult to identify a specific gene. The second strategy is a candidate gene approach for diabetic nephropathy using a case-control study and the transmission disequilibrium test (8). There are some advantages in the case-control study compared with the sib pairs analysis. Collection of DNA samples, finding a disease allele, and testing its contribution to the development of diabetic nephropathy are easier in case-control studies than in sib pairs analysis. However, positive findings in a case-control study might be attributable to unrecognized factors in the population studies (population stratification). The transmission disequilibrium test is a very good method for avoiding this effect. In the transmission disequilibrium test, affected and non-affected individuals and their parents (case and control trios) are collected. The gene verified in the case-control comparison is genotyped in trios and, using only parents who are heterozygotes for the allele, transmission of the allele to affected and unaffected offspring is determined. A transmission frequency of more than 50% to the affected offspring suggests that the specific allele increases disease susceptibility. In type 2 diabetic patients, collecting living parents is very difficult but new study designs have been proposed to examine allelic associations relying on examination of affected and non-affected individuals and their parents (9).

CASE-CONTROL STUDY IN JAPANESE WITH TYPE 2 DIABETIC NEPHROPATHY

Renin-angiotensin system

The renin-angiotensin system, which has a central role in blood pressure regulation and renal function, is not only a key regulator of sodium homeostasis, but also a modulator of vascular tone and possibly vascular structure (10). Angiotensin II production results in cell growth and matrix production in the glomeruli and interstitium. Angiotensin II is released from angiotensinogen (AGT) by the sequential action of renin and angiotensin converting enzyme (ACE). The renin-angiotensin system is generally considered to have an important role in the development of diabetic nephropathy. Ten polymorphisms in the AGT gene were reported by Jeunemaitre et al (11). Among them, the M235T (methionine to threonine substitution at codon 235) polymorphism and the G6A (G to A substitution at a position 6 base pairs upstream from the transcription starting site) were in complete linkage disequilibrium in French and Japanese populations (11). Inoue et al (12) reported that substitution of A to G at position –6 increases the basal transcription rate of the AGT gene in COS-1 cells. The increased transcription rate suggested that the A-T haplotype has higher levels of AGT in the serum and tissues and results in an increased availability of substrate for the formation of angiotensin II. Studies have shown that ACE activity is increased not only in sera but also in renal tissues in diabetic rats (13). A variant form of the ACE gene, involving intronic insertion/deletion (I/D) of a 287-base pair Alu sequence, is associated with increased plasma and tissue activity of this enzyme. Serum ACE levels are highest in patients with the DD genotype, lowest in patients with the II genotype, and intermediate in those with the ID genotype. There are two isoforms in angiotensin II receptors; one is angiotensin II receptor type 1 (AT1R) and the other is the angiotensin II type 2 receptor. The biological effect of angiotensin II in the kidney is assumed to be expressed through AT1R. Moczulski et al (14) reported that AT1R may not be implicated in the etiology of type 1 diabetic nephropathy.

Three genes, AGT, ACE, and AT1R, were thoroughly investigated in Japanese populations using case-control studies (Table 1). In 1999, the authors determined the relationship between the gene polymorphism of AGT,
Recently, the authors re-evaluated the I/D polymorphism of the ACE gene in our multicenter trial of ethnically homogeneous Japanese patients (16,24). The frequency of the DD genotype (19.7%) was slightly higher in the advanced and end stages (overt albuminuric patients with s-Cr levels of less than 1.2 mg/dL, overt albuminuric patients with s-Cr levels of more than 1.3 mg/dL, but excluding hemodialysis patients). The frequency of the DD genotype in the mild stage (normoalbuminuric and microalbuminuric patients) was 11.2%. The presence of the DD genotype increased the risk of ESRF more than that of the other genotypes. It seems that the DD genotype is associated with progression of Japanese type 2 diabetic nephropathy. This possibility now needs to be confirmed by prospective multicenter trials. Furthermore, the effects of other candidate genes or acquired factors should be examined in these patients.

Other candidate genes

Many candidate genes for diabetic nephropathy have been determined using case-control studies in Japanese populations (Table 3) (25). Endothelial nitric oxide
Genetics of type 2 diabetic nephropathy

Table 3. Other candidate genes.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Study</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>eNOS</td>
<td>Neugebauer et al (26)</td>
<td>Deletion allele in intron 4 was associated with the development of nephropathy</td>
</tr>
<tr>
<td></td>
<td>Fujita et al (27)</td>
<td>No association with nephropathy</td>
</tr>
<tr>
<td>MTHFR</td>
<td>Fujita et al (28)</td>
<td>No association with nephropathy</td>
</tr>
<tr>
<td></td>
<td>Odawara et al (29)</td>
<td>No association with nephropathy</td>
</tr>
<tr>
<td></td>
<td>Neugebauer et al (30)</td>
<td>TT genotype was associated with nephropathy</td>
</tr>
<tr>
<td>PAI-1</td>
<td>Kimura et al (31)</td>
<td>No association with the progression of nephropathy</td>
</tr>
<tr>
<td>ApoE</td>
<td>Kimura et al (32)</td>
<td>$\varepsilon_2$ Allele was protective against the progression of nephropathy</td>
</tr>
<tr>
<td></td>
<td>Eto et al (33)</td>
<td>Frequency of $\varepsilon_2$ allele was higher in patients with nephropathy</td>
</tr>
<tr>
<td>$\beta_3$-AR</td>
<td>Sakane et al (34)</td>
<td>Homozygotes for Arg64 allele were associated with nephropathy</td>
</tr>
<tr>
<td></td>
<td>Nakajima and Baba (35)</td>
<td>No association with nephropathy</td>
</tr>
<tr>
<td>ALR2</td>
<td>Ichikawa et al (36)</td>
<td>No association with nephropathy</td>
</tr>
<tr>
<td></td>
<td>Maeda et al (37)</td>
<td>No association with nephropathy</td>
</tr>
</tbody>
</table>

No association with nephropathy

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eNOS = endothelial nitric oxide synthase; MTHFR = methylenetetrahydrofolate reductase; PAI = plasminogen activator inhibitor; ApoE = Apolipoprotein E; AR = adrenergic receptor; ALR = aldose reductase.

conclusión

Epidemiological evidence strongly suggests that genetic susceptibility has a major role in the development of nephropathy in patients with type 2 diabetes. It is concluded that the DD genotype of ACE polymorphism is associated with progression of Japanese type 2 diabetic nephropathy. Genetic analysis of the corresponding chromosomal regions in humans, and examination of candidate genes residing at the loci, may provide a more thorough understanding of genetic factors involved in human type 2 diabetes. It is expected that genetic studies in this field will make dramatic progress using clinical data and technology.

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