Pathogenetic Mechanisms of Diabetic Nephropathy

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Diabetes is the leading cause of ESRD because diabetic nephropathy develops in 30 to 40% of patients. Diabetic nephropathy does not develop in the absence of hyperglycemia, even in the presence of a genetic predisposition. Multigenetic predisposition contributes in the development of diabetic nephropathy, thus supporting that many factors are involved in the pathogenesis of the disease. Hyperglycemia induces renal damage directly or through hemodynamic modifications. It induces activation of protein kinase C, increased production of advanced glycosylation end products, and diacylglycerol synthesis. In addition, it is responsible for hemodynamic alterations such as glomerular hyperfiltration, shear stress, and microalbuminuria. These alterations contribute to an abnormal stimulation of resident renal cells that produce more TGF-β1. This growth factor upregulates GLUT-1, which induces an increased intracellular glucose transport and D-glucose uptake. TGF-β1 causes augmented extracellular matrix protein deposition (collagen types I, IV, V, and VI; fibronectin, and laminin) at the glomerular level, thus inducing mesangial expansion and glomerular basement membrane thickening. However, low enzymatic degradation of extracellular matrix contributes to an excessive accumulation. Because hyperglycemia is the principal factor responsible for structural alterations at the renal level, glycemic control remains the main target of the therapy, whereas pancreas transplantation is the best approach for reducing the renal lesions.

Diabetic nephropathy is a clinical syndrome characterized by the occurrence of persistent microalbuminuria in concomitance with insulin- or non–insulin-dependent diabetes. This nephropathy has a long natural history in type 1 diabetes. Initially, the patient shows hyperfiltration, represented by high values of GFR, approximately doubling of the normal value, and occasional occurrence of microalbuminuria. The duration of these abnormal laboratory data is approximately 5 yr. Later, during a course of approximately 20 yr, the patient shows a gradual decline of the GFR and persistence of microalbuminuria that comes before mild and subsequently moderate proteinuria. The final step of the natural history of the disease is characterized by severe proteinuria with or without nephrotic syndrome and chronic renal insufficiency that declines to ESRD. The gradual impairment of the above laboratory findings is caused by structural alterations at the renal level, which at the beginning consist of a gradual and progressive accumulation of extracellular matrix (ECM) in the mesangium and glomerular basement membrane. Later, the formation of mesangial nodules represents the characteristic lesions of the Kimmelstiel-Wilson nephropathy with additional extensive tubulointerstitial lesions.

Genetics

Several factors, such as hyperglycemia, hyperlipidemia, hypertension, and proteinuria, contribute to the progression of renal damage in diabetic nephropathy. However, they are supported by a specific genetic background because only 30% of patients with type 1 and 25 to 40% of patients with type 2 diabetes develop diabetic nephropathy irrespective of glycemic control (1). In addition, the disease often involves siblings and even more so some ethnic groups.

A simple Mendelian inheritance model does not occur in diabetic nephropathy, making the approach to genetic studies very difficult. In addition, collection of DNA samples from extended pedigrees, with a lower life expectancy and old age characterizing the diseases, are often lacking. The heterogeneous clinical picture of diabetic nephropathy causes some difficulties in the identification of patients who are at high risk for disease.

The genetic background was stated many years ago by Klein *et al.* (2) in the Wisconsin epidemiologic study in which they demonstrated that metabolic control did not differ in patients with diabetes, both with and without nephropathy, and a high number of patients with diabetes did not develop the nephropathy, despite long-term, severe, chronic hyperglycemia. Familial clustering of the disease has been shown by Seaquist *et al.* (3), who reported that siblings of patients with type 1 diabetes and nephropathy have a four-fold increased risk for developing diabetic nephropathy. The ethnic background plays an important role because some races are more susceptible to diabetic nephropathy than others. In fact, the rate of developing ESRD is five times higher in relatives of black patients with type 2 diabetes in renal replacement therapy (RRT) (4). The small tribe of Pima Indians shows a high prevalence of diabetic nephropathy clusters in families with type 2 diabetes. In fact, 14% of descendants of parents with type 2 diabetes without nephropathy develop diabetic nephropathy; this percentage is higher in

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descendants of parents of whom one has proteinuria and increases in descendants of parents of whom both have diabetes and proteinuria (5). In conclusion, cumulative incidence of disease increases in the presence of parents with diabetic nephropathy. However, the occurrence of the disease is more frequent in some ethnic groups such as Pima Indians and blacks than in whites. This racial difference may be caused by specific clustering of different loci, which induces genetic susceptibility to the disease.

Diabetic nephropathy is a complex genetic disease in which more genes may be involved in developing the nephropathy. The strategy to search for genes is represented by two different approaches, namely, case–control association studies and family studies. Candidate gene studies that are based on association have rarely been successful. In fact, very contradictory demonstrations as reported by Lindner et al. (6) in a review on genetic aspects of the diabetic nephropathy have been reported in the literature. However, the familial study approach is not easy because there is no simple Mendelian inheritance model as most affected parents of the patients are dead because there is a low life expectancy. For this reason, many family studies are based on analyzing sibling pairs. The National Institutes of Health established the ongoing Family Investigation of Nephropathy and Diabetes Study Consortium to further the linkage analysis studies that led to the mapping of several susceptibility loci for diabetic nephropathy on specific regions of chromosome 3q for type 1 diabetes and on chromosome 20 and 12 for white sibling pairs with type 2 diabetes (7,8). In the Cleveland area, nephrologists collected DNA samples from multiplex diabetic families in the white and black populations (9). Then, they performed a linkage analysis of candidate genes and organized a sibling pair study design in which 212 sibling pairs who were concordant or discordant for microalbuminuria, overt proteinuria, and nephrotic-range proteinuria were included. Regions examined were located on human chromosome 10p; 10q; and at NPHS1 (nephrin), CD2AP, Wilms tumor, and NPHS2 (podocin) loci. Allele frequencies and the identity of descendent sharing were estimated separately for blacks and whites. Single-point and multipoint linkage analyses indicated that marker D10S1654 on chromosome 10p was potentially linked to diabetic nephropathy. It is interesting that the majority of the linkage evidence derived from the white sibling pairs. The investigators are now adding sibling pairs and increasing marker density on chromosome 10. Linkage with candidate regions for nephrin, CD2AP, Wilms tumor, and podocin were excluded. Therefore, a diabetic nephropathy susceptibility locus is present on chromosome 10. There are very few genetic studies in diabetic nephropathy in large multiplex pedigrees. Vardarli et al. (10) carried out linkage analysis in 18 large Turkish families (368 individuals were examined) with recurrence of type 2 diabetes and diabetic nephropathy. A logarithm of odds score of 6.1 was observed in the region of chromosome 18q22.3 to 23. This linkage was confirmed in an analysis of 101 affected sibling pairs of Pima Indians. The candidate gene in this region of chromosome 10 is ZNF 236 (Kruppel-like zinc-finger gene 236), which is glucose dependent expressed in human mesangial cells.

Pathogenesis

Resident and nonresident renal cells are stimulated by hyperglycemia in producing humoral mediators, cytokines, and growth factors that are responsible for structural alterations such as increased deposition of ECM and functional alterations such as increased permeability of glomerular basement membrane or shear stress. These alterations contribute to diabetic nephropathy. Glucose influx in the renal cells is modulated by GLUT-1, which is a surface receptor of resident renal cells. Heilig et al. (11) demonstrated that in vitro, high glucose concentrations (23 to 30 nM) induced overexpression of GLUT-1 mRNA and overproduction of GLUT-1 protein in mesangial cells. In addition, glucose transport increased in cells. GLUT-1 is modulated in its expression by TGF-β1. In fact, Inoki et al. (12) demonstrated that this growth factor modulation was dose and time dependent. When an anti–TGF-β1 monoclonal antibody was added in vitro, GLUT-1 mRNA expression and D-glucose uptake was reduced. In conclusion, endogenous TGF-β1, produced by mesangial cells cultured under high-glucose conditions, is able to enhance glucose transport to stimulate glucose uptake by inducing the overexpression of mRNA and protein GLUT-1. Thus, it accelerates glucose-induced metabolic abnormalities in mesangial cells.

Another growth factor, PDGF-β, is involved in structural alterations at the glomerular level. Di Paolo et al. (13) demonstrated in vitro downregulation of TGF-β1 in human mesangial cells in the presence of high glucose concentration and anti-PDGF BB neutralizing antibody. They evidenced that a high glucose concentration induced an early and a persistent increase of PDGF B-chain gene expression, whereas PDGF-β receptor mRNA increased by twofold after 6 h, thereafter declining after 24 h. In contrast, TGF-β1 mRNA increased after 24 and 48 h of incubation in high glucose. Therefore, they concluded that high glucose induces an early activation of a PDGF loop that in turn causes an increase of TGF-β1 gene expression, thus modulating both human mesangial cell proliferation and mesangial matrix production.

Connolly et al. (14) demonstrated that another growth factor, connective tissue growth factor, plays an important role in glomerular alteration in diabetic sclerosis because this mediator induces transient actin cytoskeleton disassembly in mesangial cells, high production of fibronectin, collagen types I and IV, and mesangial cell hypertrophy. Thus, connective tissue growth factor may be considered another therapeutic target in diabetic nephropathy. Finally, angiotensin II is an additional growth factor that stimulates resident renal cells to produce TGF-β1. Activation of the renal renin-angiotensin system and its involvement in the pathogenesis of diabetic nephropathy has been shown. In addition, angiotensin II is generated in hypertension, a disorder that frequently accompanies diabetes and accelerates progression of diabetic nephropathy. In vitro studies have shown that angiotensin II increases ECM accumulation by mesangial cells, primarily via stimulation of TGF-β expression (15,16).

Hyperglycemia is an important risk factor for the development of diabetic nephropathy. It induces an abnormal activation of protein kinase C (PKC), which is involved in the devel-

opment of diabetic nephropathy. Upregulation of PKC was observed in kidneys of rats with diabetic nephropathy (17). It was associated with TGF-β1, fibronectin, and collagen type IV upregulation. When streptozotocin-induced diabetic rats received a PKC inhibitor, LY 333531, there was a downregulation of the above growth factor and ECM proteins. The same inhibitor reduced hyperfiltration and albuminuria in rats and in mice with diabetic nephropathy (18). The identification of the susceptibility genes in diabetic nephropathy has become the focus of intensive research efforts. Among candidate genes, the PKC-β1, which encodes both βI and βII isoforms, has been chosen because an abnormal activation of PKC in diabetic patients with nephropathy has been evidenced (19,20).

Krolenski's group tested nine single-nucleotide polymorphisms (SNP) of PKC-β1 for association with diabetic nephropathy in type 1 diabetes. Both case–control and family-study designs were carried out. Allele and genotype distribution of two SNP in the promoter (−1504 C/T and −546 CG) differed significantly between patients and control subjects. These SNP were identified as a common risk haplotype for diabetic patients with duration of the diabetic state <24 yr. The risk for diabetic nephropathy was higher among carriers of the T allele of the −1540 C/T SNP and among carriers of the G allele of the −546 C/G SNP. This positive case–control study was confirmed by using the family-based transmission disequilibrium test. In fact, the T-G haplotype, with both risk alleles, was transmitted more frequently than expected from heterozygous parents to offspring, who developed diabetic nephropathy during the first 24 yr of diabetes. Therefore, DNA sequence differences in the promoter of PKC-β1 gene contribute to diseases susceptibility in type 1 diabetes (21).

Hyperglycemia is responsible for the presence of high levels of advanced glycosylation end products in patients with diabetes. These glucose metabolites stimulate intrinsic glomerular cells to produce TGF-β1, which contributes to glomerular sclerosis and tubulointerstitial damage by means of an abnormal ECM production. Forbes *et al.* (22) demonstrated that the administration of ALT 711, an advanced glycosylation end product inhibitor, in diabetic rats readily reduced the glomerulosclerosis index, the tubulointerstitial area, and albuminuria.

Hemodynamic dysfunctions in patients with diabetes are represented by blood arterial hypertension, glomerular hypertension, and hyperfiltration. Gnudi *et al.* (23) demonstrated that application of mechanical stretch to mimic a hemodynamic insult induces *in vitro* GLUT-1 overexpression and TGF-β1 production in rat mesangial cells. The presence of a monoclonal anti–TGF-β1 antibody *in vitro* reduced the GLUT-1 expression and the intracellular glucose transport. Mechanical stretch is also responsible for increased glomerular permeability to protein in patients with diabetes. Vascular permeability factor (VPF) is one of the most powerful promoters of this abnormality. Gruden *et al.* (24) studied the effect of stretch on VPF production by human mesangial cells and the intracellular signaling pathways involved. They demonstrated that the application of mechanical stretch for 6 h induced a 2.4-fold increase over control in the VPFmRNA level. Stretch-induced VPF secretion was partially prevented both by PKC inhibitor

H7 and by pretreatment with phorbol ester. The combination of both PKC and protein tyrosine kinase (PTK) inhibition completely abolished the VPF response to mechanical stretch (24) and TGFβ-1 and fibronectin production by human mesangial cells (25). In conclusion, shear stress is responsible for increased production of growth factors and ECM proteins, which contributes to mesangial cell proliferation and ECM deposition at the glomerular level.

Therapeutic Strategies

The general approach in the therapy of diabetes is represented by glycemic control, reduction of blood hypertension, lipid control, and abolishing smoking. Because hyperglycemia is the principal factor responsible for the structural alterations at the renal level, glycemic control remains the main target for therapy in patients with potential development of diabetic nephropathy. Intensive blood glucose control is the best approach in reducing the risk for microvascular complications. In addition, early treatment of blood glucose in young people with diabetes has a dramatic effect on the survival because there is an increased life expectancy (26,27). Two reports demonstrated that intensive blood glucose control with sulfonylureas or insulin reduced retinopathy, neuropathy, and cardiovascular diseases and mainly diabetic nephropathy (50%) (28,29). Gaede *et al.* (30) reported in a multifactorial intervention study a reduced risk for cardiovascular and microvascular events by approximately 50%.

Pancreas transplantation remains the best approach for the response of renal lesions in diabetic nephropathy. Fioretto *et al.* (31) demonstrated in a serial renal biopsy study that glomerular basement membrane thickness, mesangial volume, and mesangial matrix reduced gradually after 5 to 10 yr from the time of pancreas transplantation.

Acknowledgments

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