GENETICS ASPECTS OF DIABETIC NEPHROPATHY

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Abstract: Diabetic nephropathy is a clinical syndrome characterized by persistent albuminuria, a relentless decline in GFR, raised arterial blood pressure, and increased relative mortality for cardiovascular diseases. The pathogenesis of diabetic nephropathy is multifactorial, with contributions from metabolic abnormalities, hemodynamic alteration, and various growth and genetic factors.

The identification of the main genes would allow the detection of those individuals at high risk for diabetic nephropathy and better understanding of its pathophysiologyas well.The present review discusses the main information available in literature regarding some genetic variants (involved in the renin-angiotensin system, glucose and lipid metabolism and some cytoskeleton proteins) that reaffirms the importance of genetic factors in diabetic nephropathy.

INTRODUCTION

Diabetes mellitus (DM) is a set of metabolic disorders with different etiologies characterized by hyperglycemia resulting from defects in insulin secretion and/or action. In 2000, 171 million cases of DM worldwide were estimated, and that number is expected to increase to 366 million cases in 2030 (Wild *et al*., 2004).Diabetes mellitus is associated with severe complications including nephropathy, neuropathy, retinopathy and accelerated cardiovascular disease.

Diabetic nephropathy is the leading cause of end-stage renal disease (ESRD) in developed countries (The World Health Report, 2006). Evidence for a genetic component to diabetic nephropathy comes from family studies displaying familial aggregation of diabetic nephropathy both in type 1 and in type 2 diabetes mellitus (Seaquist *et al*.,1989; Pettitt *et al*.,1990; Quinn *et al*.,1996), as well as differences in the prevalence of diabetic nephropathy between ethnic groups (Nelson *et al*. 1988;Chandie *et al*., 2006).

Numerous metabolic pathways and associated groups of genes have been proposed as candidates to play a role in the genetic susceptibility to diabetic nephropathy.

STRATEGIES FOR IDENTIFICATION OF GENES ASSOCIATED WITH DIABETIC NEPHROPATHY

Genes that confer susceptibility to DN can be sought in different ways. In broad terms, there are three strategies for identifying susceptibility genes for DN: linkage analysis, population association (case-control) and the new technology Genome-Wide Association Scans (GWAS).

Linkage analysis by use of affected pedigrees is generally preferred to case-control studies but there are difficulties in establishing such a resource for DN.

Candidate genes are often analyzed in case-control studies by comparing the frequency of polymorphisms/ mutations in candidate genes among patients with and without the disease. This is an appropriate study for investigating complex genetic transmission, and it is especially useful in situations where the genetic influence is relatively low and disease-related alleles are common in a population (Adler *et al*.,2000).

Technological advances permit the genotyping of large numbers of SNPs in thousands of individualswithin the scope of a large project grant. For example, high-throughput commercial genotyping platforms such as Affymetrix (http://www.affymetrix.com) and Illumina (http://www.illumina.com) are now able to genotypeup to one million SNPs in several thousand individuals at a cost of less than GBP 1,000,000. Thus a GWAS is now a practical option for identifying nephropathy susceptibility variants, but given the still significant costs of such a scan, careful consideration must be given to the design of the study in order to maximize the chance of success (Wang *et al*., 2005).

Discovery of genetic variants that underpin susceptibility to nephropathy would permit identification of patients at risk of nephropathy shortly after diagnosis of diabetes rather than much later when persistent microalbuminuria develops, by which time there is already histological evidence of renal injury (Conway and Maxwell, 2009).

STUDIES OF CANDIDATE GENES

One approach to identify genes associated to DN is the study of candidate genes. There are many studies of candidate genes for DN but the results are inconsistent. The choice of the gene to be studied dependson knowledge concerning its actions in DN pathophysiology such as those involving blood pressure control, severity of proteinuria, insulin resistance, lipid metabolism or other pathways involved in the progression of DN. The present review discusses the main information available in literature regarding some genetic variants (involved in the RAS, glucose and lipid metabolism and some cytoskeleton proteins) that reaffirms the importance of genetic factors in diabetic nephropathy (Table 1.).

Angiotensin converting enzyme

The angiotensin-converting enzyme (ACE), a potent vasoconstrictor, catalyzes the conversion of angiotensin I to angiotensin II. It also inactivates bradykinin, a vasodilator, by bringing about its proteolysis (Crisan and Carr, 2000).

The angiotensin-converting enzyme gene (ACE) on chromosome 17q23 has repeatedly been evaluated for a role in DN. Polymorphisms in this gene are clearly associated with circulating ACE levels (Rigat *et al*., 1990) and reports suggested association between the ACE DD allele and type 1 DN (Hadjadj *et al*., 2001; Boright *et al*., 2005).

A meta-analysis including 47 studies (14 727 subjects) showed that subjects with the II genotype had a 22% lower risk of DN than carriers of the D allele [OR = 0.78, CI95% (0.69–0.88)]. Also this study support a genetic association of the ACE I/D polymorphism with diabetic nephropathy. These findings may have implications for the management of diabetic nephropathy using ACE inhibitors especially among type 2 diabetic Asians (Ng *et al*., 2005). Although a large meta-analysis failed to confirm the DN association in white individuals (Kunz *et al*., 1998) a recent report (Hadjadj *et al*., 2007) from the European Rational Approach for the Genetics of Diabetic Complications (EURAGEDIC) Study Group detected evidence for association of several ACE polymorphisms (including the "D" deletion allele) in a large case-control study, with somewhat consistent findings in a family-based transmission disequilibrium testing analysis.

In summary, it appears that polymorphisms in the ACE gene may have a role in the progression of DN, rather than in the susceptibility to it. Thus, evaluating the ACE I/D polymorphism is by no means a reliable and cost-effective tool to identify patients at risk and those who may benefit the most of renoprotective therapy with ACE inhibitors or angiotensin II antagonists and, possibly, with other inhibitors of the RAS, such as renin and aldosterone antagonists.

Angiotensinogen and angiotensin II receptor type 1

Other variants in the renin–angiotensin system that were also widely studied and reproduced, such as the rs699 variant of AGT and the rs5186 polymorphism of AGTR1, were not associated with diabetic nephropathy in a meta-analysis (Mooyaart *et al*., 2011).

Aldose reductase

Aldose reductase is an important enzyme in the polyol pathway, and is suggested as contributing to diabetic microangiopathic complications. Several mechanisms have been proposed to explain how AKR1B1 activity leads to hyperglycaemia-induced lesions in different tissues (Chung and Chung , 2003)

Ko *et al.*were the first to identify seven alleles at the locus of the (AC)n dinucleotide repeat sequence upstream of AKR1B1. The most common allele contains 24 (AC) repeats and was named Z (Ko *et al.,* 1995). Several studies have demonstrated a correlation between the Z-2 allele (23 repeats) and susceptibility to an increased risk of DN in both T1DM and T2DM (Shah *et al*., 1998; Olmos *et al*., 1999; Moczulski *et al*., 2000; Neamat-Allah *et al*., 2001; Liu *et al*., 2002)

A second AKR1B1 polymorphism has been observed at position−106 of its promoter region. This C106T polymorphism was identified in both Caucasian and Asian subjects with T1DM or T2DM, and association with DN has been observed. Sivenius *et al.* (2004) and Gosek *et al.* (2005) suggested that this polymorphism could be involved in the early development of microalbuminuria in Finnish T2DM patients and is a risk factor for development of DN in T2DM patients with poor glycaemic control, respectively. All these results suggest that AKR1B1 polymorphisms play a role in DN development.

Glucose transporter 1

The glucose transporter 1 (SLC2A1, also know as GLUT1) is the major representative of the family of facilitative glucose transporters that are expressed in glomerular, mesangial, endothelial cells and podocytes. SLC2A1 is likely to be pivotal in raising intracellular glucose levels by activating pathogenic pathways (Koya and King, 1998; Larkins and Dunlop, 1992; Vlassara,1997).

Several works have tried to determine whether SLC2A1 might be a candidate gene conferring susceptibility to DN. In a study Ng *et al*., (2002) confirms that SNPs at the GLUT1 (XbaI -intron 2 and HaeIII SNPs -exon 2) are associated with susceptibility to diabetic nephropathy in type 1 diabetes. Although these SNPs confer a considerable

personal risk for diabetic nephropathy, they account for a limited proportion of cases among type 1 diabetic patients. A meta-analysis concluded that there is, indeed, a significant association between the SLC2A1 *XbaI* polymorphic site and DN, but larger studies are needed (Zintzaras and Stefanidis, 2005)

Apolipoprotein E

The apolipoprotein E gene (APOE) on chromosome 19q has also been associated with susceptibility to type 1 DN (Araki *et al*., 2000) and type 2 DN (Hsu *et al*., 2005). APOE is a polymorphic protein that consists of three isoforms, E2, E3, and E4, encoded by the alleles ε2, ε3, and ε4, which are defined by a single amino acid substitution at two sites (reviewed by Mahley and Rall, 2000). The E2 allele is thought to lead to an increased risk of diabetic nephropathy and the E4 allele is thought to have a protective effect. Both the E2 and the E4 allele were associated with diabetic nephropathy in a meta-analysis (Mooyaart *et al*., 2011)

Adionectin

Adiponectin is an adipocytokine that is produced by fat cells (Guzik *et al*., 2006). Circulating levels of adiponectin are reduced in individuals who are obese and have diabetes, and levels rise after weight loss. Positive correlations have been reported between circulating high molecular weight adiponectin level and HDL cholesterol, with negative correlations among circulating inflammatory markers, triglycerides, and homeostasis model assessment of insulin resistance. Adiponectin gene (ADIPOQ) polymorphisms reportedly play a protective role in susceptibility to coronary heart disease (Qi *et al*., 2006). With clear relationships between atherosclerosis and DN, ADIPOQ is likely a gene that may play a role in both vascular processes. In a recent analysis, rs17300539 of ADIPOQ, which is believed to mitigate vascular damage, was not associated with diabetic nephropathy (Mooyaart *et al.,* 2011)

Peroxisome proliferator activated receptor gamma 2

Peroxisome proliferator activated receptor gamma 2 (PPARγ2) is the predominant adipose isoform of this receptor and is expressed selectively in the adipose tissue where it modulates the expression of target genes implicated in adipocyte differentiation and glucose homeostasis. PPARγ2 is considered, therefore, a major candidate gene for T2DM and/or obesity and, recently, for type 2 DN. Three studies have evaluated its association with type 2 DN. In the study by Herrmann *et al*. (2002) the Pro12Ala polymorphism was associated with lower albumin excretion rates among Ala12 carriers with type 2 DN, which may indicate a protective effect of this allele. These findings were confirmed by Caramori *et al*. (2003). More recently, Pollex *et al*. (2007) showed that the Ala12 allele carriers have reduced occurrence of microalbuminuria (1.5-fold reduction of the albumin/creatinine ratio). All these results indicate a protective correlation between the Ala12 polymorphism and the albumin excretion rate. The mechanism underlying the protective effect of the Ala12 allele is yet unknown.

Adducins

Adducin (ADD) is a heterodimeric cytoskeleton protein composed of α, β and γ-subunits. These proteins are encoded by three genes (ADD1, ADD2 and ADD3) that map to different chromosomes. ADD genes show a similar gene structure, suggesting their deviation from a single gene that has undergone duplications and rearrangements during evolution. In humans, the ADD1 is widely expressed, while the ADD2 is especially expressed in neuronal, renal and erythropoetic tissue (Matsuoka *et al*., 2000).

The α-subunit regulates the activity of transmembrane ion pumps and is encoded by the adducing 1 (ADD1) gene, located in chromosome 4q21.

In a large study that investigate the role of the α-adducin gene in genetic susceptibility to diabetic nephropathy, Conway *et al*., (2004) have found no evidence of association between variation in the α-adducin gene and the development of nephropathy in the Irish population. While they cannot exclude the possibility of a minor gene effect, it is unlikely that common variation within the α-adducin gene plays a major role in the genetic predisposition to diabetic nephropathy in Irish population. Another study of the same group investigated the ADD2 gene and their results suggest that common polymorphisms and putatively functional variants in the ADD2 gene do not strongly influence genetic susceptibility to diabetic nephropathy in the White population studied with type 1 diabetes (Currie *et al*., 2008). Most studies on the role of adducins were performed on hypertensive patients and less on diabetic nephropathy. Recently Lanzani *et al*., described an epistatic interaction between the adducing gene (ADD1 and ADD3) in a large cohort of never treated hypertensive individuals. Patients who carried both the mutated ADD1 Trp allele and ADD3 G/G had the higher systolic and diastolic blood pressure values (p=0.002). (Lanzani *et al*., 2005). So far there are a small number of studies on the effect of adducins in the patology and progression of diabetic nephropathy.

Table 1. Candidate genes associated with type 1 diabetic nephropathy

Class	Gene name	Symbol	Location
Renin-angiotensin system	Angiotensin-converting enzyme 1	ACE 1	17q23
	Angiotensinogen	AGT	1q42-43
	Angiotensin II receptor	AGT1	3q21-25
Glucose metabolism	Aldose reductase	AKR1B1	7q35
	Glucose transporter-1	SCL2A1	1p35
Lipid metabolism	Apolipoprotein E	APOE	19q13.2
	Adiponectin	ADIPOQ	3q27
	Peroxisome proliferator activated receptor gamma 2	PPARγ 2	3p25
Cytoskeletal genes	Alfa adducin	ADD1	4p16.3
	Beta adducin	ADD2	2p13.3
	Gamma adducin	ADD3	10q25.2

CONCLUSION AND PERSPECTIVES

Due to the growing burden of the management of diabetes and its complications, it is important to identify DN predictors, in order to facilitate its diagnosis and treatment. Identification of risk genes could provide a powerful tool for identifying the subset of patients who have diabetes and will progress to nephropathy and ESRD. Early identification will facilitate earliar intervention, ultimately delaying and reducing the impact of DN.

Most genetic studies have been performed in selected populations but they are heterogeneous between them. It should also be pointed out that an isolated candidate gene is sought when various genes are probably involved and possibly interlinked (Carpena *et al*., 2010). Joint efforts are essential to achieve robust findings in the study of genetics of DN. In the light of remarkable advances in this area of study, we hope that in the near future patients at high risk for developing DN could be identified and benefited with earlier specific therapies. Hence, by combining the expertise of geneticists and clinicians, there is real hope that the genetic basis of diabetic nephropathy and other complex renal diseases may be unravelled offering new opportunities for screening and therapeutic intervention.

In addition, new pharmacogenomic developments will contribute to better treatment choices for DN and, more importantly, will help preventing it based on an individual's genetic characteristics.

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