Genetics of diabetic nephropathy

Bases genéticas da nefropatia diabética

Mariana P. Carpena1, Dimitris V. Rados1, Denise A. Sortica1, Bianca M. de Souza1, André Fernandes Reis2, Luis Henrique Canani1,2, Daisy Crispim1,2

SUMMARY
The increasing prevalence of diabetes mellitus has led to a growing number of chronic complications including diabetic nephropathy (DN). In addition to its high prevalence, DN is associated with high morbidity and mortality especially due to cardiovascular diseases. It is well established that genetic factors play a role in the pathogenesis of DN and genetically susceptible individuals can develop it after being exposed to environmental factors. DN is probably a complex, polygenic disease. Two main strategies have been used to identify genes associated to DN: analysis of candidate genes, and more recently genome-wide scan. Great efforts have been made to identify these main genes, but results are still inconsistent with different genes associated to a small effect in specific populations. The identification of the main genes would allow the detection of those individuals at high risk for DN and better understanding of its pathophysiology as well.

Keywords
Nephropathy; diabetes mellitus; genetics; genetic predisposition

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 (1). Concomitantly, the number of patients who develop chronic complications of DM including diabetic nephropathy (DN) has grown.

Chronic kidney disease (CKD) is defined as renal damage characterized by structural or functional abnormalities of the kidneys or glomerular filtration rate (GFR) < 60 mL/min/1.73 m², with or without renal damage, over a period of at least three months, regardless of its causes (2). DN is characterized by a set of dia-
betic pathophysiological changes, which begin with glo-
merular hyperfiltration and renal hypertrophy, and then
progress to proteinuria and GFR reduction. Based on
the levels of urine albumin excretion, in a didactic man-
ner, DN has two phases: incipient nephropathy or the
microalbuminuria phase with urine albumin excretion
(UAE) ranging between 20-199 µg/min (or 30-300
mg/24h); and, clinical nephropathy or the proteinuria
phase with UAE > 199 µg/min (> 300 mg/24h) or
proteinuria ≥ 500 mg/24h. Microalbuminuria is con-
sidered a risk factor for DN progression (3).

About 20% to 30% of patients with type 1 and type 2
DM develop DN; however, a smaller proportion of pa-
tients with type 2 DM will progress to end-stage renal
disease (ESRD). Due to its high prevalence, the majority
of patients requiring dialysis are type 2 DM. DN is the
most common cause of ESRD in several countries (4,5)
but not all diabetic individuals will develop this com-
pliation. Those who do not develop DN in the first 15
years after disease onset seem to be genetically protected
(6). Many environmental factors have been established
as contributing to the development of DN while the role
of others has yet to be clearly understood (7). It is known
that factors such as hyperglycemia, arterial hypertension
and/or dyslipidemia play a role in the development of
DN in genetically predisposed individuals only.

The rates of hospitalization for all causes are about
three times higher in patients with CKD than in those
who do not have the disease (8). According to Pagano
and cols. (9), patients with type 2 DM with DN and pe-
ripheral arterial disease are 1.2 to 1.3 times more likely
to be hospitalized (9). Besides its high disease burden,
ESRD is associated with increased mortality, mainly due
to cardiovascular causes (10). Reduced renal function is
by itself an indicator of high mortality. Other concomi-
tant risk factors such as hypertension and autonomic
neuropathy can contribute to cardiovascular diseases
(10). Even patients with DN initially characterized by
microalbuminuria already have an increased risk for car-
diovascular diseases and higher mortality (11). Accord-
ing to accumulating evidence the risk of developing
DN and cardiovascular disease starts when UAE values
are still within normal ranges (12). Some authors con-
sider albuminuria a major risk factor for cardiovascular
events rather than just a simple marker of DN progres-
sion (13) as some patients develop fatal cardiovascular
events even before showing reduced renal function. In
the metropolitan area of Porto Alegre, southern Brazil,
25% of new patients require dialysis due to DM, and
they show a high mortality rate during the first two
years on dialysis (14).

It has not yet been clearly established by which
mechanism some diabetic patients will progress to-
wards loss of renal function and require dialysis while
others will maintain normal renal function. Research
has focused on seeking potential genetic alterations
associated to CKD and ESRD. In fact, genetic evidence
has been found in case-control association and linkage
studies, and more recently using genome-wide scan
(GWS). These studies support the assumption that on-
set, progression, and severity of DN can be in part at-
tributed to genetic factors (15).

The identification of genes associated with DN will
allow recognizing those individuals who are at high
risk of developing this complication. It will also allow a
better understanding of the mechanisms and progress
of DN. Earlier and more aggressive therapies could be
provided to high-risk individuals and thus reduce the
associated high disease burden and mortality. Advances
in pharmacogenetic research may help treatment choices
by selecting renoprotective drugs according to indi-
vidual haplotypes (16).

The present review discusses the main information
available in literature that reaffirms the importance of
genetic factors in DN. Some findings of our recent ge-
netic studies are also summarized.

GENETIC TRANSMISSION MODELS

The genetic transmission mode of DN is still controver-
sial. Theoretically, as in other diseases, it might occur in
three distinct forms, which would lead to the develop-
ment of DN (6,17).

Monogenic form: mutations in a gene with a domi-
nant role.

Oligogenic form: mutations/polymorphisms in a
few genes would contribute in an independent and cu-
mulative manner to increase susceptibility.

Polygenic form: alterations in many DNA loci, and
each would have a small and cumulative effect on DN
development.

Considering that DN is a multifactorial disease, the
mode of transmission is likely to be polygenic, and ge-
netic interaction with other environmental factors and
clinical data such as duration of the DM, arterial hyper-
tension, dyslipidemia, smoking would lead to the de-
velopment of DN. Familial aggregation studies do not
allow to clearly establish a mode of transmission but
they provide evidence supporting that this is a polygen-
ic complex disease (6,17). On the other hand, studies using segregation analysis (18,19) suggest that familial aggregation of DN is due mainly to the action of a main genetic locus. This effect would be consistent with a monogenic or oligogenic form where few genes would have greater effect on the phenotype.

Since several genes may be involved in the development of DN and many of them have not yet been identified, it is not possible to be completely sure about the exact heritability pattern in most cases.

**STRAATEGIES FOR IDENTIFICATION OF GENES ASSOCIATED WITH DIABETIC NEPHROPATHY**

Genes that confer susceptibility to DN can be sought in different ways. A widely established method is the candidate gene approach. The search for candidate genes includes the study of polymorphisms in one or more genes potentially involved in the pathogenesis of the disease. This approach is useful even when the influence of a gene on disease development is small (17).

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 (17). However, this approach is very sensitive to population stratification, which may lead to spurious associations. In the light of that it has been proposed that these studies should include a large sample to obtain very small p-values and be based on well-established a priori assumptions. This approach has allowed to describing many polymorphisms associated with DN; however, the study results have been inconsistent. One of the most studied alterations is insertion/deletion on the angiotensin-converting enzyme (ACE) gene. A meta-analysis has shown that the D allele is associated with high risk of DN (20).

Another approach used to analyze candidate genes is the transmission disequilibrium test (TDT). This approach is not influenced by population stratification, but information is required about the individuals studied and their parents, and only heterozygote parents are informative. The frequency of transmission of risk allele is compared to the expected 50%. Its main limitation is having access to the individual and his/her parents, especially for type 2 DM that has a late onset in life. In a case-control study we demonstrated that the ectoenzyme nucleotide pyrophosphate phosphodiesterase (ENPP1) gene is associated with early development of ERSD in patients with type 1 DM (21), and using the TDT approach we confirmed this association was not due to population stratification (21). Araki and cols. (22) used the same approach to demonstrate that the APOE-epsilon 2 allele is associated with DN in type 1 diabetic individuals (22).

More recently candidate genes are being tested in prospective studies. This study design is less prone to survival bias than case-control studies but they are expensive and time-consuming. Alternatively, the authors are studying cohorts that have been followed up over a long period of time (23). The limitation of these studies is that they are not specifically designed to address a genetic effect of a specific gene.

The use of microarrays has enabled fast, accurate analysis of a large number of candidate genes and to perform GWS based on single nucleotide polymorphisms (SNPs). GWS can identify chromosomal regions that contain genes potentially involved in the genesis of a disease under study. Panels of microsatellite markers or SNPs, at ~10 centimorgans (cM) intervals throughout the genome, are genotyped in several generations of families of patients with DM, affected or not with DN. The markers that most commonly occur in family members affected with DN indicate the location of a functional variant associated with the disease and is in linkage disequilibrium with the marker. Given the difficulty of finding large families with several members affected by DN, an alternative approach is to compare the observed and expected frequency of the markers in pairs of diabetic siblings concordant and discordant for DN. A major advantage over the candidate gene approach is that this approach can detect chromosomal regions containing genes that were not previously known to be implicated in the pathogenesis of DN. However, it has the disadvantage of only detecting genes that have a moderate or large effect (24). Using GWS we identified three polymorphisms located on chromosomes 9q and 11p associated with DN in two different populations of patients with type 1 DM (25).

**EVIDENCE FOR GENETIC PREDISPOSITION TO DIABETIC NEPHROPATHY**

**Familial aggregation of diabetic nephropathy**

Studies of familial aggregation have showed that some families are predisposed to DN (26-28). Studies on sib-
lings with type 1 or type 2 DM have reported that DN in one of the siblings is associated with around a 3- to 4-fold increase in the risk of DN in the other sibling (29,30).

There appears to be a genetic inheritance contributing to the development of CKD. Forsblom and cols. (31) showed that the heritability (h²) of UAE rate is approximately 30% when analyzing non-diabetic children of type 2 diabetic individuals (31). This finding was corroborated by another study, which found that after adjustment for covariables such as sex, age, obesity and DM, approximately 30% of the variability of albumin-creatinine rate was due to genetic factors (32).

The magnitude of the familial association cannot be attributed only to exposure to similar risk factors, suggesting there is a genetic component (33).

**Genes associated with diabetic nephropathy and different phenotypes**

although proteinuria and loss of renal function often occur concomitantly, there is evidence of different genetic predispositions for each condition (15). This can explain why some patients may have persistent proteinuria without progressing to loss of renal function (34) and other patients have loss of renal function without proteinuria or microalbuminuria (15,35).

In genetic association studies, it is very important to clearly define the phenotype of interest. Most studies on heritability use albuminuria or proteinuria as a DN marker. In fact, loss of renal function measured by GFR is strongly associated with increased UAE. However, it is important to point out that a significant proportion of patients with DM develop loss of renal function but they maintain normoalbuminuria (35-37) and, conversely, some patients with clinical proteinuria maintain a stable GFR over the years (38).

UAE and GFR reduction are both genetically determined, but apparently independently (15). Langefeld and cols. (39) evaluated 310 families comprising 662 patients with type 2 DM and found an h² of 0.35 for UAE, and of 0.69 for GFR (39). These findings were similar to those described in other studies reporting an h² of creatinine depuration of 0.63 among mono- and dizygotic twins (40).

**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 (Table 1). The choice of the gene to be studied depends on knowledge concerning its actions in DN pathophysiology such as those involving blood pressure control, severity of proteinuria or insulin resistance (41). Below we present our experience with this approach.

Type 2 DM patients with microalbuminuria have high serum levels of circulating fatty acids (50) compared to normoalbuminuric patients. Intestinal absorption of long-chain fatty acids is controlled by the intestinal fatty acid-binding protein 2 (FABP2). Thus, alterations in the gene that codifies the FABP2 can be candidates indicating predisposition to DN. A54T polymorphism (rs1799883) in FABP2 is associated with altered protein conformation, leading to greater affinity of the FABP2 protein for intestinal fatty acids with consequent serum increase. We genotyped this polymorphism in 1,042 Brazilian patients with type 2 DM. An association of the T allele was found at different stages of DN (Figure 1) (48). This association was replicated in an independent sample of 483 white American subjects with type 2 DM (48).

Several studies analyzed the insertion/deletion (I/D) polymorphism in the ACE gene but the results about its association with DN in patients with type 1 and type 2 DM were inconsistent, possibly due to ethnic differences of the populations studied (20). Besides, several studies used longer duration of DM as an inclusion criterion, which might predispose to survival bias, insofar as the possible genes associated with DN could also be associated with increased mortality. Therefore, we chose to investigate a potential association between I/D polymorphism in the ACE gene and the development of DN in 982 Brazilian patients with type 2 DM, taking into account the duration of their disease. In patients with 10 years or less of disease, having an allele D (DD/ID) resulted in an odds ratio (OR) of 2.66 (95% CI 1.12-6.58, p = 0.015) for incipient DN and 3.19 (95% CI 1.18-9.30, p = 0.012) for overt DN. On the other hand, in patients with longer disease duration no increased risk for DN was seen associated with allele D (45).

Gene candidates for insulin resistance can also be considered DN candidates since insulin resistance is a common characteristic of patients with type 1 and type 2 DM who present increased UAE (51,52). The polymorphism in the ENPP1 gene, previously known as PC-1, was found to be associated with insulin resistance (53). Based on this finding, a case-control study was conducted to assess the association between advanced DN and K121Q polymorphism (rs1805101)
Table 1. Candidate genes for diabetic nephropathy

<table>
<thead>
<tr>
<th>Author</th>
<th>Gene</th>
<th>Polymorphism</th>
<th>OR (95% CI)</th>
<th>p-value</th>
<th>Case definitions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Canani and cols., 2002</td>
<td>ENPP1/PC-1</td>
<td>K121Q (rs1805101)</td>
<td>2.3 (1.2-4.4)</td>
<td>0.014</td>
<td>Patients with type 1 DM with ESRD</td>
</tr>
<tr>
<td>Leitao and cols., 2008</td>
<td>ENPP1/PC-1</td>
<td>K121Q (rs1805101)</td>
<td>1.06 (0.73-1.54)</td>
<td>0.830</td>
<td>Type 2 DM with micro- or macroalbuminuria</td>
</tr>
<tr>
<td>Ng and cols., 2002</td>
<td>GLUT1</td>
<td>XbaI enhancer-2</td>
<td>1.83 (1.01-3.33)</td>
<td>0.044</td>
<td>Patients with type 1 DM with persistent proteinuria or ESRD</td>
</tr>
<tr>
<td>Santos and cols., 2006</td>
<td>CATALASE</td>
<td>-262C/T (rs35448603)</td>
<td>0.92 (0.63-1.37)</td>
<td>0.776</td>
<td>White patients with type 2 DM with micro- or macroalbuminuria or ESRD</td>
</tr>
<tr>
<td>Canani and cols., 2005</td>
<td>ACE</td>
<td>Ins/Dei polymorphism</td>
<td>2.66 (1.12-6.58)</td>
<td>0.015</td>
<td>Type 2 DM with less than 24 years of disease: incipient and overt DN, respectively</td>
</tr>
<tr>
<td>Santos and cols., 2005</td>
<td>CYBA</td>
<td>p22phox C242T (rs4673)</td>
<td>2.24 (1.15-4.37)</td>
<td>0.045</td>
<td>White patients with type 2 DM, smokers, with micro- or macroalbuminuria or ESRD</td>
</tr>
<tr>
<td>Santos and cols., 2005</td>
<td>RAGE</td>
<td>-429C (rs1800625)</td>
<td>1.31 (0.62-2.76)</td>
<td>0.720</td>
<td>703 Brazilians with type 2 DM (520 Caucasians) and 183 of African ascendance with micro- or macroalbuminuria or ESRD</td>
</tr>
<tr>
<td>Canani and cols., 2005</td>
<td>FABP-2</td>
<td>A54T (rs1799883)</td>
<td>2.4 (1.1-5.4)</td>
<td>0.005</td>
<td>Type 2 DM with microalbuminuria or persistent proteinuria or ESRD</td>
</tr>
<tr>
<td>Caramori and cols., 2003</td>
<td>PPARY2</td>
<td>Pro12Ala (rs1801282)</td>
<td>0.465 (0.229-0.945)</td>
<td>0.034</td>
<td>Type 2 DM with CKD and normoalbuminuria</td>
</tr>
</tbody>
</table>

CKD: Chronic kidney disease; DM: diabetes mellitus; DN: diabetic nephropathy; ESRD: end-stage renal disease; OR: odds ratio; CI: confidence interval.

Figure 1. Odds ratio (♦) with 95% confidence intervals (dotted line) for the presence of A54T polymorphism in FABP2 at the different stages of renal involvement adjusted for diabetes duration, body mass index, arterial hypertension, HbA1c, and cholesterol levels. ESRD: end-stage renal disease.

in the ENPP1 gene of patients with type 1 DM. The proportion of patients with the variant Q was 21.5% in the control group, 31.5% in those with proteinuria, and 32.2% in those with ESRD (p = 0.012). In a stratified analysis for DM duration (< 24 or ≥ 24 years of DM), the risk of early onset of ESRD for patients with the variant Q was 2.3 times greater than for those without the variant Q (95% CI 1.2-4.4) and it was not associated with late onset ESRD (21). This association was confirmed using TDT, which showed that this association was not due to population stratification (21).

Polymorphisms in GLUT1 gene associated with DN were also examined. GLUT1 is a glucose transporter in the kidneys and it has been associated with early kidney alterations leading to proteinuria. We studied 230 controls (patients with DM1, disease duration of at least 15 years and normoalbuminuria), and 262 cases (151 patients with persistent proteinuria and 111 with ESRD). The homozygosis for the XbaI(-) allele was associated with a discrete increased risk for DN when compared to other genotypes combined [OR 1.83 (95% CI 1.01-3.33)]. A significant difference in genotype distribution among cases and controls was seen for the enhancer-2 SNP1 (p = 0.036). There was an excess of genotype AA among the cases (10.7%) compared to controls (4.8%). These homozygous individuals presented an increased risk for DN compared to AG and GG genotypes combined [OR 2.38 (95% CI 1.16-4.90)] (43).

Other studies analyzed different genes and did not find an association with DN (Table 1). One of these studies showed that, when patients were stratified for smoking, allele T (p22phox C242T polymorphism; rs4673) was more frequently seen in smokers with ESRD or persistent proteinuria than in normoalbuminuric patients (43% vs 32%; p = 0.045). The multiple logistic regression analysis confirmed that CT and TT genotypes were independently associated with a greater risk of overt DN among smokers (OR = 6.76; 95% CI 1.83-25.02) (46).

Our experience with candidate genes allowed us to identifying some genes that can be related to the development and severity of DN.
Genome-wide scan studies

Recent GWS studies have demonstrated chromosomal regions potentially associated with DN. Table 2 shows the studies using a GWS approach. In DN concordant Pima Indians, a potential association was seen between chromosome 7q and phenotype of DN. The area between two markers (D7S500 and D7S1804), in 144 cM, showed the strongest association, with a LOD score of 2.04. Two other susceptibility loci were also found in chromosomes 20 (LOD score of 1.83) and 3 (LOD score of 1.48) (54).

Genes located in chromosomes 22q, 5q, and 7q might be involved in the determination of UAE severity in patients with and without DM (58). In a genome-wide association study, regions in chromosomes 19 and 2q were identified as associated with proteinuria and ESRD in patients with type 1 DM (60). A locus in chromosome 1q was associated with ESRD only, while a locus in chromosome 20p was associated with proteinuria only (60). Two independent loci were identified in chromosome 3q, 59 cM from each other, one associated with proteinuria and the other with ESRD (60).

A genome-wide association study with 360,000 SNPs using the microarray Affymetrix 5.0 was recently conducted in two independent cohorts of Caucasians patients with type 1 DM (25). SNPs that were highly significant in the two cohorts were selected for further analyses. Eleven SNPs located at 4 loci were closely associated with DN (p < 1 x 10^{-5}) (Table 3). Some of these associations found in the cross-sectional study were confirmed in a prospective sample of the Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications (DCCT/EDIC). Three of the 11 initial SNPs had their association confirmed, two were borderline and the remaining did not show a significant association with the development of DN (proteinuria or CKD) (25) (Table 4).

Other authors have reported an association of different chromosomal regions using GWS approach to search for DN related genes (Table 2) (55-57,59).

**CONCLUSION AND PERSPECTIVES**

In conclusion, clinical and epidemiological studies have evidenced a genetic component of DN. However, no specific gene has been able to explain most DN cases yet. The results of studies that identified genes or genome regions associated with DN were quite inconsistent. The lack of more consistent results is probably due to different factors. 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. Joint efforts are essential to achieve robust findings in the study of genetics of DN. In the light of remarkable

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**Table 2.** Genome-wide scan studies for genes associated with diabetic nephropathy

<table>
<thead>
<tr>
<th>Author</th>
<th>Population studied</th>
<th>Regions associated</th>
<th>Phenotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>Imperatore and cols., 1998 (54)</td>
<td>Pima Indians- 98 siblings with DM2, concordant for ND</td>
<td>7q, 3 and 20</td>
<td>Albuminuria or proteinuria</td>
</tr>
<tr>
<td>Vardarli and cols., 2002 (55)</td>
<td>125 patients with DM2</td>
<td>18q22.3-23</td>
<td>Albuminuria</td>
</tr>
<tr>
<td>Tanaka and cols., 2003 (56)</td>
<td>Japanese with DM2 grouped into cases (with DR and DN) and controls (with DR and without DN)</td>
<td>16q13</td>
<td>Albuminuria</td>
</tr>
<tr>
<td>Bowden and cols., 2004 (57)</td>
<td>266 siblings with DM2, African-Americans, concordant for DN</td>
<td>3q, 7p and 18q</td>
<td>CKD</td>
</tr>
<tr>
<td>Krolewski and cols., 2006 (58)</td>
<td>59 Caucasian families, 1 African-American, and 3 Hispanic. Members with and without DM2</td>
<td>22q, 5q and 7q</td>
<td>Albuminuria</td>
</tr>
<tr>
<td>Iyengar and cols., 2007 (59)</td>
<td>1,227 subjects from 378 families with DM1 or DM2, concordant or discordant for DN</td>
<td>2q14.1, 7q21.1 and 15q26.3</td>
<td>Albuminuria or ESRD</td>
</tr>
<tr>
<td>Rogus and cols., 2008 (60)</td>
<td>Sibling concordant for DM1 and discordant for ND</td>
<td>19q, 2q and 3q</td>
<td>Proteinuria and ESRD</td>
</tr>
<tr>
<td>Pezzolesi and cols., 2009 (25)</td>
<td>Type 1 DM patients with DN (cases) and without DN (controls)</td>
<td>7p - CPVL/CHN2</td>
<td>Proteinuria and ESRD</td>
</tr>
</tbody>
</table>

CKD: Chronic kidney disease; DM: diabetes mellitus; DN: diabetic nephropathy; DR: diabetic retinopathy; ESRD: end-stage renal disease.
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. We also expect to see a consequent reduction in disease burden and mortality due to this serious complication. 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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