Genetic Factors in Diabetic Nephropathy

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Several genes that predispose to type 2 diabetes have recently been identified. In addition to the recognized and powerful effects of environmental factors, there is abundant evidence in support of genetic susceptibility to the microvascular complication of nephropathy in individuals with both type 1 and type 2 diabetes. Familial aggregation of phenotypes such as end-stage renal disease, albuminuria, and chronic kidney disease have routinely been reported in populations throughout the world, and heritability estimates for albuminuria and glomerular filtration rate demonstrate strong contributions of inherited factors. Recent genome-wide linkage scans have identified several chromosomal regions that likely contain diabetic nephropathy susceptibility genes, and association analyses have evaluated positional candidate genes under these linkage peaks. These complimentary approaches have demonstrated that polymorphisms in the carnosinase 1 gene on chromosome 18q, the adiponectin gene on 3q, and the engulfment and cell motility gene on 7p are likely associated with susceptibility to diabetic nephropathy. Additional genes that seem to be of importance in renal phenotypes include manganese superoxide dismutase and angiotensin 1-converting enzyme, with nitric oxide synthase implicated in albuminuria. This article reviews the inherited aspects of diabetic kidney disease with particular emphasis on recently implicated genes and pathways. It seems likely that the risk for diabetes-associated kidney disease is magnified by inheriting risk alleles at several susceptibility loci, in the presence of hyperglycemia.

and nonprofit), and questionnaires were completed by the local nursing and social worker staffs. This large study confirmed the results of the single-center reports. An even larger percentage of individuals with ESRD likely have relatives who either have clinically silent nephropathy that has not yet progressed to need for dialysis or died from cardiovascular causes related to chronic kidney disease before starting dialysis (7). A recent US population-based sample (8) detected association between serum levels of C-reactive protein and family history of ESRD while confirming the effects of ethnicity, gender, and cause of renal disease.

Urinary albumin excretion is a hallmark of DN; however, albuminuria can fluctuate widely, even fully resolve, and is a stronger predictor of cardiovascular disease (CVD) events than progressive nephropathy. Heritability estimates for albuminuria (urine albumin: creatinine ratio) and kidney function (GFR) allow for the estimation of genetic and environmental contributions to DN. A heritability (h²) = 1 suggests the presence of a Mendelian disorder (e.g., autosomal dominant polycystic kidney disease), whereas h² = 0 reveals the contribution of environmental factors. Heritability estimates for albuminuria were significant, ranging from 0.3 to 0.44 in Finnish (9), New England (10), and southeastern US (11) families enriched for members with type 2 diabetes, and the estimate was higher (h² = 0.49) in Hypertension Genetic Epidemiology Network (HyperGEN) families enriched for multiple siblings with hypertension (12). Similarly, GFR was significantly heritable in families with diabetes and hypertension, ranging from 0.36 to 0.75 (11,13,14). These results are impressive, considering that albuminuria and GFR change during the course of DN. Thus, familial aggregation of these intermediate phenotypes solidifies the concept that DN susceptibility genes exist. Environmental factors seemed to be minor contributors to these relatively high h² estimates. Two segregation analyses in multiplex families with members who had type 2 diabetes suggested that major gene effects likely contribute to the development of DN (15,16). Quinn et al. (17) reported that an autosomal dominant major gene with a common disease allele was likely present.

In addition, familial aggregation of renal histology is observed in siblings with diabetes, independent of diabetes duration and disease control (18); a greater prevalence of hypertensive parents among children with diabetes and nephropathy are seen (19); and ethnic differences in susceptibility to DN exist (20). Together, these observations support genetic contributions to DN. These epidemiologic studies set the stage for genome-wide searches to detect chromosomal regions that harbor DN-susceptibility genes.

### Genetic Approaches to Identifying DN Genes

In tandem with advances in the epidemiology of DN, options for gene identification in common complex diseases have rapidly evolved with completion of the Human Genome Project and an improved understanding of the haplotype block structure of our genome. Broad categories of molecular genetic approaches include linkage and association studies, with recent advances in association analysis including mapping by admixture disequilibrium (MALD) (21) and whole-genome association (WGA) studies (22).

Linkage studies are family based and evaluate coincident inheritance of polymorphic genetic markers that are relatively uniformly distributed throughout the genome with the presence or absence of disease among relatives. In DN, families have often been limited to sibling pairs or two-generation families because of the late age at onset of DN. Initial linkage studies were performed using microsatellite repeat genetic markers; however, single-nucleotide polymorphisms (SNP) are now commonly used with equal or better information content at lower cost. Because we receive one copy or allele of a given gene (or a given polymorphic genetic marker) from each parent, among full siblings, 50% are expected to share one (of two) alleles at a given locus, 25% share both alleles, and 25% share neither allele; overall allele sharing is at 50%. In siblings who are discordant for DN (affected sibling pairs) and are tested for sharing of a polymorphic marker that is in linkage disequilibrium with a nearby DN locus, analysis of large numbers of sibling pairs would demonstrate that significantly more than 50% of affected sibling pairs share the linked genetic marker. In contrast, siblings who are discordant for DN (discordant sibling pairs) are expected to demonstrate significantly less than 50% sharing of the polymorphic marker that is linked to the nearby DN disease locus. Chromosomal regions linked with DN in families (e.g., linkage peaks) presumably contain DN susceptibility genes or are false-positive results. Genome-wide linkage analyses provide a model-free screen of the entire genome; however, linkage is generally regarded as less sensitive compared with association analyses (see below, Table 2) but does have the capability of identifying genomic regions that harbor multiple genes or multiple variants with small effect sizes, in addition to the simpler scenario of a single strong contributor to risk.

Because of the need to recruit large numbers of informative families, linkage studies are difficult, expensive, and time-consuming. Association analyses using unrelated case patients and control subjects are simpler and often far less expensive. Association analysis approaches are used for detecting disease genes (or markers that are in linkage disequilibrium with causative genes) but may be subject to false-positive results because of population stratification. The important capability of association analyses is the ability to detect relatively modest effect sizes (e.g., good power to detect odds ratios (OR) of 1.3 to 1.5 or less in 500 case patients and 500 control subjects). It should be noted that even with this approach, success depends on the existence of a relatively common risk allele. Family-based association studies, also called family-based transmission disequilibrium testing (TDT), tend to reduce the potential bias of population stratification. Recently, it has become possible to perform association analyses in large numbers of case patients with DN and control subjects with diabetes and without nephropathy using 500,000 (or more) SNP markers spaced across all chromosomes, a WGA analysis. This approach will become even more powerful in the near future with technologies using upward of 1 million SNP markers for association analysis. WGA remains an expensive undertaking, but several groups have DN studies planned or under way.
Ethnic differences in disease course, such as occur in DN, can suggest the presence of causative genetic factors. Genetic admixture between two populations results from the sharing of alleles that were initially far more common in one ethnic group. For example, black and European American admixture has resulted in approximately 20% admixture of “European alleles” in the black population. A novel technique to detect association, MALD, tests for association of ethnic-specific genetic markers that were more common in one ethnic group (either the one with the higher or the lower disease prevalence) and are then disproportionally transmitted into affected members in the other ethnic group (21). MALD is potentially useful in situations in which ethnic differences in disease incidence exist between recently admixed populations. Examples of each of these techniques as applied to DN are described next.

Genome-Wide Linkage Scans for DN
Six complete genome-wide linkage scans have been published in type 2 DN, with one in type 1 DN (and another partial genome scan in type 1 DN) (14,23–29). Although most of these analyses evaluated small numbers of families from different ethnic groups, several consistent regions of linkage have been detected (Table 1). In type 1 DN, two reports identified linkage to markers on chromosome 3q in white families (27,28), and this location was subsequently replicated in a family-based TDT analysis (30). A 3q peak was also observed in a type 2 DN genome scan in black families (23), in the same region but distal to the white peak. Several regions of linkage have been replicated among the various groups with type 2 DN, as well. The 18q22–23 linkage region was replicated in four populations (23,26,29), the 7q35–36 peak in three reports (25,26,31), and the 7p15 and 10q26 peaks in two each (14,23,32). Positional candidate genes for DN in these regions are now being sought and identified (Table 2).

Chromosome 3q Linkage Peak in Type 1 DN
Intensive efforts have been undertaken to identify the causative gene under the 3q linkage peak in type 1 DN. The nearby angiotensin II type 1 receptor gene was quickly excluded from involvement (27). Vionnet et al. (33) subsequently evaluated 14 candidate genes for type 1 DN on chromosome 3q in an association analysis using a combined population of 1057 unrelated Danish, Finnish, and French case patients with type 1 DN, 1127 control subjects with diabetes and without nephropathy, and 532 trio families. In an initial association analysis, polymorphisms in three genes—glucose transporter 2, kininogen, and adiponectin—were nominally associated with type 1 DN, including genes that were initially far more common in one ethnic group. Attempts at replication using TDT testing in trios confirmed significant association in the adiponectin gene (ADIPOQ) with excess transmission of the A allele in the promoter to individuals with DN and of the G allele to those without DN (P = 0.011). This suggests that polymorphisms in ADIPOQ may be involved in DN susceptibility. The pooled odd ratio for the ADIPOQ Prom 2GA allele was 1.46 (95% confidence interval 1.11 to 1.93; P = 0.006), with results driven predominantly from the Danish (P = 0.011) and French (P = 0.071) cohorts.

Adiponectin is an adipocytokine that is produced by fat cells (34). 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 polymorphisms reportedly play a protective role in susceptibility to coronary heart disease (35). It is thought to have antiatherogenic properties via downregulation of expression of adhesion molecules on endothelial cells through inhibition of NF-κB activation, reduction of endothelial oxidative stress and proliferation while stimulating nitric oxide synthase activity, and reduction of vascular smooth muscle cell proliferation by inhibiting the effects of a number of growth factors (34).

TDT and Microsatellite Association Analysis in Type 1 DN
Ewens et al. (30) tested 115 candidate genes for association with type 1 DN using TDT. This was a comprehensive analysis of a large number of potential candidate genes that previously were implicated in the pathogenesis of DN. The power of this study was limited by the inclusion of only 72 trio families. Several genes were nominally associated with type 1 DN, including genes in the broad categories of extracellular matrix material (COL4A1, LAMA4, and LAMC1), matrix metabolism (TIMP3 and MMP9), transcription factors/signaling molecules (HNF1B1/TCF2, NRPI, PKRCB1, SMAD3, and USP1), growth factors/growth factor receptors (IGFI, TGFBR2, and TGFBR3), and those that are believed to be important in the regulation of kidney function (AGTR1, AQP1, BCL2, CAT, GPX1, LPL, and p22phox).

McKnight et al. (36) performed a novel genome-wide fluorescence-based DNA microsatellite association screen in 400 Irish individuals with diabetes (200 case patients with type 1 DN and 200 control subjects without nephropathy). This “low-resolution” WGA study used 6000 microsatellite markers and pooled DNA samples from case patients and control subjects. The top 50 most consistently associated marker pools were identified, and the samples that composed the pools were individually genotyped. Two markers on chromosome 10q (D10S558 and D10S1435) were significantly associated with type 1 DN, and four additional markers demonstrated trends toward association (D6S281, D4S293, D2S291, and D17S515). Of note, the 10q association with D10S1435 was previously observed in nondiabetic ESRD in black individuals (37).

Type 2 DN
To date, six complete genome-wide scans (GWS) have been published in type 2 DN. The largest are the interim and final reports from the National Institute of Diabetes and Digestive and Kidney Diseases-sponsored Family Investigation of Nephropathy and Diabetes (FIND) (26,38,39). Although these GWS were performed in different ethnic groups, most con-
Table 1. Summary of results from genome-wide linkage scans for diabetic nephropathy a

<table>
<thead>
<tr>
<th>Chromosome</th>
<th>Region</th>
<th>Maximum LOD</th>
<th>Population</th>
<th>Study</th>
<th>Phenotype</th>
<th>Characteristics</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>3q</td>
<td>13</td>
<td>4.55</td>
<td>Black Sibling pairs</td>
<td>Type 2 DN</td>
<td>Age at ESRD onset</td>
<td>(23)</td>
<td></td>
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<td></td>
<td>21.3</td>
<td>2.67</td>
<td>Finnish Discordant sibling pairs</td>
<td>Type 1 DN</td>
<td></td>
<td>(28)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>25.1</td>
<td>3.1</td>
<td>White Discordant sibling pairs</td>
<td>Type 1 DN</td>
<td></td>
<td>(27)</td>
<td></td>
</tr>
<tr>
<td>7q</td>
<td>12.3</td>
<td>1.84</td>
<td>West African Sibling pairs</td>
<td>Type 2 DN</td>
<td>ACR</td>
<td>(24)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>21.1</td>
<td>(6.0 × 10^{-4})</td>
<td>White 90% sibling pairs</td>
<td>Predominantly type 2 DN</td>
<td>CC</td>
<td>(26)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>21.3</td>
<td>(6.0 × 10^{-5})</td>
<td>Black 90% sibling pairs</td>
<td>Predominantly type 2 DN</td>
<td>Nephropathy</td>
<td>(26)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>33</td>
<td>2.04 to 2.73</td>
<td>Pima Indian Sibling pairs</td>
<td>Type 2 DN</td>
<td></td>
<td>(25)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>36.2</td>
<td>3.1</td>
<td>(99 cM) 94% white Families</td>
<td>Type 2 DN</td>
<td>ACR</td>
<td>(31)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(1.1 × 10^{-4}) White 90% sibling pairs</td>
<td>Predominantly type 2 DN</td>
<td>Nephropathy</td>
<td>(38)</td>
<td></td>
</tr>
<tr>
<td>7p</td>
<td>21.3</td>
<td>4</td>
<td>94% white Sibling pairs</td>
<td>Type 2 DN</td>
<td>CC-GFR</td>
<td>(14)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>32.1</td>
<td>3.59</td>
<td>Black Sibling pairs</td>
<td>Type 2 DN</td>
<td>Age at diabetes onset</td>
<td>(23)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(12 cM)</td>
<td>(1.6 × 10^{-4})</td>
<td>American Indian 90% sibling pairs</td>
<td>Predominantly type 2 DN</td>
<td>ACR</td>
<td>(38)</td>
<td></td>
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<td>(78 cM)</td>
<td>(1.0 × 10^{-3})</td>
<td>Mexican American 90% sibling pairs</td>
<td>Predominantly type 2 DN</td>
<td>GFR</td>
<td>(39)</td>
<td></td>
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<tr>
<td>10q</td>
<td>23.31</td>
<td>3.1</td>
<td>94% white Sibling pairs</td>
<td>Type 2 DN</td>
<td>Diabetic/nondiabetic; CC-GFR</td>
<td>(14)</td>
<td></td>
</tr>
<tr>
<td>18q</td>
<td>26</td>
<td>2.47</td>
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<td>Type 2 DN</td>
<td>Age at ESRD onset</td>
<td>(32)</td>
<td></td>
</tr>
<tr>
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<td>22.1</td>
<td>3.72</td>
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<td>Type 2 DN</td>
<td>Age at diabetes onset</td>
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<tr>
<td></td>
<td>22.1</td>
<td>(3.15 × 10^{-2})</td>
<td>White Discordant sibling pairs</td>
<td>Predominantly type 2 DN</td>
<td>Nephropathy</td>
<td>(26)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>22.3–23</td>
<td>6.1</td>
<td>Turkish Families</td>
<td>Type 2 DN</td>
<td></td>
<td>(29)</td>
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</tbody>
</table>

aP values are used where logarithm of odds (LOD) scores were not reported. ACR, albumin-to-creatinine ratio; CC, creatinine clearance; DN, diabetic nephropathy; eGFR, estimated GFR.

bChromosome band (map location).
<table>
<thead>
<tr>
<th>Gene Class</th>
<th>Gene</th>
<th>Location</th>
<th>Loci</th>
<th>Population</th>
<th>Phenotype</th>
<th>Reference</th>
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<tr>
<td>Cytokines and growth factors</td>
<td>Adiponectin</td>
<td>3q</td>
<td>ADIPOQ</td>
<td>Danish, Finnish,</td>
<td>Type 1 DN</td>
<td>(33)</td>
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<td></td>
<td>IGFl-1</td>
<td>12q23.2</td>
<td>IGF1</td>
<td>White</td>
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<td>(30)</td>
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<td></td>
<td>IGF-binding protein 1</td>
<td>7p14</td>
<td>IGFBP1</td>
<td>White</td>
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<td>(51)</td>
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<td>TGF-β receptor II</td>
<td>3p24.1</td>
<td>TGFβR2</td>
<td>White</td>
<td>Type 1 DN</td>
<td>(30)</td>
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<tr>
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<td>TGF-β receptor III</td>
<td>1p22.3</td>
<td>TGFβR3</td>
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<td>Collagen type IV, α 1</td>
<td>7q32.1</td>
<td>COL4A1</td>
<td>White</td>
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<td>(30)</td>
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<td>Laminin, α 4</td>
<td>6q21</td>
<td>LAMA4</td>
<td>White</td>
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<td>Laminin, γ 1</td>
<td>1q25.3</td>
<td>LAMCI</td>
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<td>Matrix metalloproteinases and dipeptidases</td>
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<td>Tissue inhibitor of metalloproteinase 3</td>
<td>22q12.3</td>
<td>TIMP3</td>
<td>White</td>
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<td>(30)</td>
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<td>Matrix metalloproteinase 9</td>
<td>20q13.12</td>
<td>MMP9</td>
<td>White</td>
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<td>(30)</td>
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<td>Carnosinase</td>
<td>18q22.3</td>
<td>CNDP1</td>
<td>White</td>
<td>Type 2 DN</td>
<td>(41,42)</td>
</tr>
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<td>Transcription factors</td>
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<td>HNF1B1/transcription factor 2, hepatic (MODY5)</td>
<td>17q12</td>
<td>HNF1B1/TCF2</td>
<td>White</td>
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<td>Neuropilin 1</td>
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<td>NRPI</td>
<td>White</td>
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<td>(30)</td>
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<td>Protein kinase C β 1</td>
<td>16p12.1</td>
<td>PRKCB1</td>
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<td>(30)</td>
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<td>SMAD, mothers against DPP homolog 3</td>
<td>15q22.33</td>
<td>SMAD3</td>
<td>White</td>
<td>Type 1 DN</td>
<td>(30)</td>
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<td>Upstream transcription factor 1</td>
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<td>USFI</td>
<td>White</td>
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<td>(30)</td>
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<td>Renal function and renin system</td>
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<td>Angiotensin II receptor, type 1</td>
<td>3q24</td>
<td>AGTR1</td>
<td>White</td>
<td>Type 1 DN</td>
<td>(30)</td>
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<td>Aquaporin 1</td>
<td>7p14.3</td>
<td>AQP1</td>
<td>White</td>
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<td>(30)</td>
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<td>B-cell leukemia/lymphoma 2 (bcl-2)</td>
<td>18q21.33</td>
<td>BCL2</td>
<td>White</td>
<td>Type 1 DN</td>
<td>(30)</td>
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<td>Catalase</td>
<td>11p13</td>
<td>CAT</td>
<td>White</td>
<td>Type 1 DN</td>
<td>(30)</td>
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<td>Glutathione peroxidase 1</td>
<td>3p21.3</td>
<td>GPX1</td>
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<td>(30)</td>
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<tr>
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<td>Lipoprotein lipase</td>
<td>8p21.3</td>
<td>LPL</td>
<td>White</td>
<td>Type 1 DN</td>
<td>(30)</td>
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<tr>
<td></td>
<td>Cytochrome b, α polypeptide</td>
<td>16q24.3</td>
<td>p22phox</td>
<td>White</td>
<td>Type 1 DN</td>
<td>(30)</td>
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<tr>
<td></td>
<td>Angiotensin-converting enzyme</td>
<td>17q23</td>
<td>ACE</td>
<td>White</td>
<td>Type 1 DN, Type 2 DN</td>
<td>(60,61,64,65)</td>
</tr>
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<td></td>
<td>Inflammatory factors</td>
<td></td>
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<td>Engulfment and cell motility factor</td>
<td>7p14</td>
<td>ELMO1</td>
<td>Japanese, Black</td>
<td>Type 2 DN</td>
<td>(23,46–48)</td>
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<td>Nitric oxide synthase 3</td>
<td>7q36.1</td>
<td>NOS3</td>
<td>Japanese, White</td>
<td>DN, Type 1 DN</td>
<td>(54–58)</td>
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<td>Superoxide dismutase 2</td>
<td>6q25</td>
<td>SOD2</td>
<td>Caucasian, Korean, Japanese</td>
<td>Type 1 DN, Type 2 DN</td>
<td>(66–68)</td>
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<td></td>
<td>Lipid metabolism</td>
<td>19q</td>
<td>ApoE</td>
<td>White</td>
<td>Type 1 DN, Type 2 DN</td>
<td>(69,70)</td>
</tr>
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</table>

References:
Chromosome 18q22–23: The Carnosinase 1 Gene

The chromosome 18q22–23 linkage peak in type 2 DN demonstrated a logarithm of odds score >6 (roughly corresponding to \( P < 10^{-6} \)) in 18 large Turkish families who lived in Germany (29). Although not identified in an initial GWS in Pima Indians, markers in the 18q region were found to be linked in this population as well. Subsequently, the 18q linkage was confirmed in black sibling pairs with diabetic ESRD and in members of the National Institute of Diabetes and Digestive and Kidney Diseases–supported FIND in the first-phase GWS, which contained 1227 individuals in 378 families (linkage in the FIND was predominantly driven by the European American and American Indian families). This consistent evidence for linkage strongly supported the existence of a type 2 DN gene(s) and 306 healthy control subjects (42). Similar to the report by Janss et al. (41), 38.6% of healthy subjects, 36.8% of patients with type 2 diabetes and without nephropathy, and 26.1% of patients with definite DN were 5L-5L homozygotes (OR for definite DN versus type 2 diabetes without nephropathy 1.65; \( P = 0.02 \)). Yard (43) developed reporter assays for the five, six, and seven leucine CNDP1 variants that were expressed in Cos-7 cells and determined the amount of secreted protein. The five leucine-expressing Cos-7 cells secreted significantly less carnosinase than the others, demonstrating that the hydrophobic leucine stretch is of critical importance for targeting this protein into the secretory pathway. Taken together, these studies strongly support a role for the carnosinase gene CNDP1 in susceptibility to type 2 DN. Carnosinase is a scavenger of oxygen free radicals, inhibits formation of advanced glycosylation end products, and inhibits TGF-\( \beta \) production (44). Individuals who are CNDP1 5L-5L homozygotes secrete less carnosinase and are therefore expected to have higher carnosine levels with protection from DN. The role of CNDP1 in DN among black individuals is less clear: There is no evidence of 5L protection; however, other CNDP1 alleles may influence risk (45).

Chromosome 7p14: The Engulfment and Cell Motility 1 and IGF-Binding Protein 1 Genes

Shimazaki et al. (46) performed a gene-centric WGA in unrelated Japanese individuals who had type 2 DN and diabetes without nephropathy. A SNP in the 18th intron of engulfment and cell motility 1 (ELMO1) on chromosome 7p14 was strongly associated with DN, and the region of association extended 100 kb upstream and downstream from this SNP. The 7p region was also linked with susceptibility to type 2 diabetes in 638 black affected sibling pairs from 247 multiplex families who had type 2 diabetes and each had a proband with advanced DN (47), in a subset of 266 affected sibling pairs who were concordant for diabetic ESRD from 166 of these families (23) and in FIND families (26).

Because ELMO1 is a large gene, Shimazaki et al. (46) genotyped an additional 516 polymorphisms in 640 Japanese DN case patients and 426 control subjects. Several of these additional markers were also associated, the strongest being the +9170 A/G SNP (GG versus AG + AA; OR 2.67; \( P = 0.0008 \)). In situ hybridization revealed increased gene expression of ELMO1 in diabetic mouse kidney tissue; increased expression of ELMO1 was seen in COS cell lines that were exposed to a hyperglycemic environment, and these cells overexpressed extracellular matrix protein genes (collagen type 1 and fibronec- tin) and underexpressed metalloproteinase genes. Our analyses confirm a role for this gene in susceptibility to both diabetes and ESRD in black individuals (48), although a single report in white individuals failed to replicate this association (49).

Previously, ELMO1 was known to play a role in phagocytosis of dying cells and is required for cell migration and changes in cell shape. Maeda’s group (50) in Japan subsequently demonstrated that ELMO1 expression was increased in an animal model of chronic glomerulonephritis, resulting in overexpression of extracellular matrix proteins and loss of cell adhesion properties; hence, ELMO1 could plausibly play a role in the development of several glomerular disorders, including DN.

Another positional candidate gene on 7p14 that has been...
implicated in susceptibility to type 2 DN is IGF-binding protein 1 (IGFBP1). Stephens et al. (51) evaluated 732 patients with type 2 diabetes in the Salford Diabetes Register. Nearly 25% of the male and 12% of the female participants had DN. Several polymorphic SNP were associated with a reduced prevalence of nephropathy, and haplotype analysis revealed that 97% of the genetic variation for IGFBP1 in the population sample could be accounted for using two “renoprotective” SNP. One of these SNP may affect the tissue delivery of IGF-1 by IGFBP1, because this SNP is in exon 4 near the integrin-binding RGD motif. The RGD motif is believed to be important in extracellular matrix interactions mediated by the binding of integrin-α5β1. It is possible that both of these genes on 7p are involved in susceptibility to type 2 DN.

Chromosome 7q35: Endothelial Nitric Oxide Synthase
Endothelial nitric oxide synthase (NOS3) is a positional and functional candidate gene for DN that is located on chromosome 7q. NOS3 regulates systemic endothelial function by maintaining endothelial-dependent vasodilation, including in the renal microcirculation. NO is synthesized from L-arginine by NOS. In rodent models, long-term NO blockade leads to elevated glomerular capillary pressure with proteinuria and glomerulosclerosis (52). The T-786C polymorphism is known to reduce NOS3 gene promoter activity and has been associated with coronary artery disease and DN (53). It is possible that the NOS3 association is with albuminuria, in and of itself a potent CVD risk factor, relative to risk for development of progressive DN (54–58).

Additional Candidate Genes of Interest
In addition to the genes described in the previous sections, a wide range of biologically relevant genes have been assessed for association with DN. A summary of salient observations is provided in Table 2. For obvious reasons, there has been long-standing interest in whether genes in the renin-angiotensin-angiotensin axis were involved in propensity toward renal failure or whether gene polymorphisms could provide useful markers to predict medical response to therapy (pharmacogenomics). Unfortunately, many such studies were performed with inadequate interrogation of the genes (e.g., too few SNP evaluated) and contained small numbers of cases and controls. 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 (59), and reports suggested association between the ACE DD allele and type 1 DN (60–62). Although a large meta-analysis failed to confirm the DN association in white individuals (63), a recent report (64) 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 TDT analysis. The results were strongest among the French cohort and clearly weaker in the Finnish sample, suggesting heterogeneous effects among different ethnic groups. In addition, Ng et al. (65) evaluated three tagging markers (A-5466C, T-3892C, and Ins/Del) at the ACE locus for disease association with type 2 DN. Although none of these markers was associated with DN in isolation, haplotype analysis revealed that nearly twice as many of the DN cases (13.6%) inherited the A-5466C, T-3892C, Deletion (ATD) “risk” haplotype, compared with diabetes non-nephropathy controls (7.5%; P = 0.009). This report further suggested that haplotypes at the ACE locus may be associated with DN.

Manganese superoxide dismutase (MnSOD) or SOD2 on 6q25 has been implicated in risk for type 2 DN on the basis of the role of oxidative stress on renal cell injury. A valine/alanine polymorphism in the targeting sequence of the SOD2 gene (rs4880) seems to affect the efficiency with which MnSOD is transported across the mitochondrial matrix. Association of the Val/Val genotype of SOD2 with type 2 DN was initially reported in Korean (66) and Japanese (67) cohorts. This genotype was then associated with type 1 DN in European cigarette smokers, in whom inheritance of the Val/Val genotype was associated with a significant 32% increase in overall risk for DN (68). In the highest risk group of smokers who inherited the Val/Val genotype, the risk for DN was >2.5 times greater than in the low-risk group. In vitro studies demonstrate that the valine polymorphism (relative to alanine) results in less efficient transport of SOD, potentially compromising the ability of the cell to neutralize superoxide radicals and placing susceptible individuals at risk for oxidative stress, albuminuria, and resulting mesangial expansion and glomerulosclerosis.

The apolipoprotein E gene (APOE) on chromosome 19q has also been associated with susceptibility to type 1 DN (69) and type 2 DN (70). ApoE is a polymorphic protein that consists of three isoforms, E2, E3, and E4, encoded by the alleles e2, e3, and e4, which are defined by a single amino acid substitution at two sites (reviewed by Mahley and Rall [71]). These substitutions have been shown to alter the affinity of ApoE for its receptors, thereby influencing lipid metabolism. The e4 allele has previously been associated with increased risk for both Alzheimer’s disease and CVD. A number of studies have investigated associations between the ApoE isoforms and DN; however, the results remain inconclusive. This may be due in part to the small sample size used in all but one study (70). Several studies (72–74) concluded that the ApoE4 isoform is associated with protection from type 2 DN in both white and Japanese individuals. Hsu et al. (70) corroborated this finding, demonstrating that the frequency of the e4 allele is decreased in type 2 diabetes and chronic kidney disease and revealing association between the e2 allele and chronic kidney disease, a result that is consistent with several older studies of type 1 DN in white individuals (75,76) and one of type 2 DN in Japanese individuals (77). In contrast, an equal number of studies (78–80) failed to find any association between e2 and type 2 DN or microvascular complications associated with type 2 diabetes. Given the significance of ApoE in lipid metabolism, the mechanism behind associations between ApoE polymorphisms and DN is likely to be complex.

Conclusions
There is now a consensus that genes contribute to risk for DN. Earlier investigations that focused on genetic mapping and
analysis of specific candidate genes provided the foundation for current studies that target linkage peaks and specific genes. As our knowledge of DN (and other complex disorders) increases, the sophistication of approaches and size and cost of these studies have also increased. These efforts are beginning to identify genes that have increasingly compelling evidence for association. Recent successes in identifying diabetes and other genes through linkage or WGA methods suggest that we may be at the threshold of an exciting era for understanding the genetic risk factors underlying DN. It is noteworthy that a second report of a DN gene, plasmacytoma variant translocation gene PVT1, identified using a WGA approach, has appeared (81).

There are still challenging issues in this area of research. Emerging evidence suggests that patients with type 2 diabetes likely have differing causes of disease and that different ethnic groups may have variable risk associated with a specific gene. If this proves to be true, then efforts in minority populations will need to be increased. Finally, the importance of finding DN groups may have variable risk associated with a specific gene.

Disclosures
None.

References


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