Genetic Susceptibility Contributes to Renal and Cardiovascular Complications of Type 2 Diabetes Mellitus

Barry I. Freedman, Donald W. Bowden, Michèle M. Sale, Carl D. Langefeld, Stephen S. Rich

More than 100,000 Americans were diagnosed with end-stage renal disease (ESRD) and initiated renal replacement therapy in 2003. Diabetes mellitus (DM) was the etiology of ESRD in nearly one half (44.2%) of these incident cases. Overall, 324,826 Americans received renal replacement therapy in 2003. Diabetes mellitus (DM) was the etiology of ESRD compared with whites.1 Diabetic subjects are not at equal risk for developing progressive diabetic nephropathy (DN).2 Diabetic blacks have a 4-fold higher incidence rate of ESRD compared with whites.1

Familial aggregation of albuminuria, DN, and ESRD have long been observed.3,4 The magnitude of familial aggregation of ESRD was demonstrated in a study of 25,883 incident dialysis patients.5 After exclusion of cases with monogenic genetic kidney diseases (ie, autosomal dominant polycystic kidney disease and hereditary nephritis), almost one quarter (22.8%) of incident dialysis patients (31.6% of black women and 27.5% of black men) reported having a first-degree and/or second-degree relative with ESRD. Multivariate analysis revealed that diabetes-associated ESRD, black ethnicity, and younger age at ESRD were significant and independent predictors of familial aggregation of ESRD. A population-based cohort study6 further demonstrated that the familial clustering of DN was in excess of that which could be explained by an excessive prevalence of diabetes and hypertension in families. Together, these reports suggest the presence of “renal failure susceptibility genes,” independent from genetic factors causing type 2 diabetes per se.

Genetic Factors in DN

Many studies have evaluated the roles of specific “candidate genes” in susceptibility to DM-ESRD and DN. The results of these candidate gene analyses have often been contradictory. Discrepancies between studies are likely because of small sample sizes, significant association caused by population stratification, and/or inadequate evaluation of polymorphisms in candidate genes (ie, interrogation of too few single nucleotide polymorphisms [SNPs] for complete coverage of the haplotype block structure of the candidate gene).

A powerful alternative approach, the “genome-wide linkage scan,” represents a comprehensive genetic survey of the entire genome for regions coinherited with (or linked to) a trait. The genome-wide linkage scan uses linkage analysis of genetic markers (evenly) spaced over all of the chromosomes in collections of families containing multiply affected individuals. Because the genome-wide linkage scan targets “anonymous” markers (microsatellites or SNPs) for linkage, there is no assumption of markers being related to specific pathways or underlying knowledge of disease biology. The genome-wide linkage scan is technically simple yet difficult in terms of time and expense for the collection of samples. The collection of cases and controls for the evaluation of individual candidate genes is easier than the collection of families, yet the linkage approach has the advantage of being able to comprehensively survey the genome and locate new, potentially undiscovered genes. The limitation of the genome scan approach is that whereas it usually has the power to detect major genetic effects, it does not usually have the power to detect loci with small effects.

Four complete genome-wide linkage scans have been published in DN, evaluating Pima Indian families,7 large multigenerational Turkish kindreds,8 black ESRD-affected sib pairs (ASPs),9 and large white families.10 Imperatore et al7 identified evidence for the linkage to DN on chromosome 7q35 and suggestive evidence for linkage on 3q26, 9q22, and 20p12 in 98 type 2 DM-affected Pima ASPs. The endothelial NO synthase gene (NOS3) is located within the 7q linkage peak making it a positional candidate gene for DN. Several reports have identified association between DN and either the T-786C SNP or an intron 4 insertion/deletion polymorphism in NOS3,11–14 whereas other studies have not.15,16

Vardarli et al8 evaluated 18 large, multigenerational Turkish families with type 2 DN and identified strong evidence for linkage on 18q22.3-23 (log of the odds score=6.1). Evaluation of this region in Pima families confirmed evidence for linkage, although this region was not identified in their original genome-wide linkage scan. Evidence for linkage to type 1 DN was detected on 3q in a partial genome scan in whites; however, the angiotensin II type 1 receptor gene (ATR1) was excluded as containing the causal variant.17 A genome-wide linkage scan for albuminuria in 59 extended white pedigrees enriched for members with type 2 DM revealed evidence for linkage to 22q, 5q, and 7q. Additional
Evidence for linkage to 21p was observed when the analyses were restricted to DM-affected relative pairs.\textsuperscript{10}

A genome-wide linkage scan was performed in 206 black ASPs with advanced DN or ESRD from 166 families.\textsuperscript{9} Ordered subsets analysis and nonparametric linkage regression interaction analysis were used to analyze more homogeneous groups of families for linkage. These results confirmed linkage of DN to 3q and 18q, with the 3q linkage most pronounced in families with early ages at ESRD onset and the 18q linkage strongest in families with the earliest age at onset of DM. Linkage on chromosome 10q was detected as suggested in earlier reports.\textsuperscript{18} This result was confirmed in partial genome scans\textsuperscript{19,20} and in a genome-wide association scan.\textsuperscript{21} Replication for linkage on 7p was demonstrated for families with the lowest BMI. Plausible DN susceptibility genes under the 7p peak include the engulfment and cell motility 1 gene (\textit{ELMO1}) on 7p14.2-14.1, as well as the nearby insulin-like growth factor binding proteins 1 (\textit{IGFBP1}) on 7p14-p12 and 3 (\textit{IGFBP3} on 7p14-12). \textit{ELMO1} was associated with DN in Japan\textsuperscript{22} and \textit{IGFBP1} in the United Kingdom City of Salford Diabetes Archive.\textsuperscript{23}

A linkage between glomerular filtration rate and markers on 2q36 and albuminuria and markers on 15q12 was detected previously in Mexican-American families enriched for type 2 DM.\textsuperscript{24,25} The Strong Heart Family Study recently replicated evidence of linkage of albuminuria to chromosomes 3q and 10q and nephropathy to 18q in Native Americans (A. Mottl, personal communication, March 2006).

In a search for positional candidate genes under the chromosome 18q peak, Janssen et al\textsuperscript{26} detected significant evidence for association between DN and the carnosine dipeptidase I gene (metalloproteinase M20 family; \textit{CNDP1}) in European whites from Germany, the Netherlands, and Prague and Arabic individuals from Qatar. Individuals homozygous for 5 copies of a trinucleotide repeat sequence in exon 2 (encoding 5 leucine residues 5L-5L) were at a 2.56-fold reduced risk for DN when compared with all other genotypes. The 5L-5L homozygotes were designated as having the protective “\textit{CNDP1 Mannheim}” allelic variant. This protective variant was associated with lower serum carnosinase levels. Our group replicated the association between 5L homozygosity in \textit{CNDP1} and protection from type 2 diabetic ESRD in white Americans (B.I. Freedman, personal communication, April 2006). In vitro experiments further revealed that the addition of carnosine to culture media inhibited podocytes from producing type VI collagen and fibronectin and mesangial cells from producing transforming growth factor-$\beta$ in response to high-glucose environments. These results strongly suggest that carnosine and the carnosinase pathway are important determinants of DN susceptibility. It is expected that identification of other DN genes will be identified under the other peaks, particularly in genomic regions with confirmatory evidence for linkage.

A genome-wide linkage scan in $>$4295 individuals of white, Hispanic-American, Native-American, and black ethnicity (1500 families in the Family Investigation of Nephropathy and Diabetes [FIN]) is currently underway at the Center for Inherited Disease Research.\textsuperscript{27} The FIND family study encompasses 1740 DN-ASPs and 900 DN-discordant sib pairs and has adequate power to detect areas of linkage to DN within each ethnic group and shared regions of linkage between ethnicities. The Genetics of Kidneys in Diabetes (GoKIND) has also recruited large numbers of families and singletons for the detection of genes underlying type 1 DN (http://www.gokind.org/access/home.html).

**Epidemiology of Diabetes-Associated Cardiovascular Disease**

Many studies have demonstrated that both coronary and carotid artery diseases cluster in families. In white type 2 diabetic families from the Diabetes Heart Study (DHS), familial aggregation (defined by heritability [h\textsuperscript{2}]) was shown for coronary artery calcified plaque ([\textit{CorCP}] h\textsuperscript{2}=0.40) and carotid artery intima medial thickness (IMT; h\textsuperscript{2}=0.41).\textsuperscript{28,29} Peyser et al\textsuperscript{30} also reported a high heritability in white families for \textit{CorCP} and for carotid IMT. Together, these studies suggest that there is a powerful genetic component to subclinical cardiovascular disease (CVD).

In the general population, diabetic blacks clearly suffer disproportionate morbidity and mortality from coronary artery disease, relative to whites. In contrast, given equal access to health care, blacks have far lower rates of clinical coronary artery disease. Karter et al\textsuperscript{31} assessed whether access to healthcare was associated with ethnic disparities in complications in 62,432 diabetic individuals insured by Kaiser Permanante. The adjusted black:white hazard ratio for myocardial infarction was 0.56 (\textit{P}<0.001). Youn et al\textsuperscript{32} detected a significant 49\% lower rate of coronary disease in blacks relative to whites in a longitudinal cohort study of 429,918 diabetic subjects cared for by the Veterans Administration. Similarly, black patients performing renal replacement therapy have improved overall and CVD mortality compared with whites, despite later referral to nephrologists and more severe hypertension.\textsuperscript{33–35} Once on dialysis, all patients have equal access to physicians and typically qualify for health insurance through the Centers for Medicare and Medicaid Services.

Blacks are clearly at greater risk for ischemic strokes than whites. However, marked ethnic variability exists in the frequency of stroke subtypes. The majority of reports demonstrate the propensity for blacks to have increased intracranial atherosclerosis (small vessel disease), whereas whites tend to have increased extracranial atherosclerosis (large vessel carotid artery disease),\textsuperscript{36–41} although minor differences have been noted.\textsuperscript{42–44} When differences in access to care are minimal, as in our institution, 97.3\% (292 of 300) of consecutive carotid endarterectomies performed between 2001 and 2004 were in whites. Even allowing for subtle differences in access to healthcare or invasive procedures, this difference suggests that extracranial carotid artery disease is far more severe in whites. Large vessel renal artery stenosis also seems to occur more often in whites relative to blacks.\textsuperscript{45}

As in clinical coronary and extracranial carotid artery disease, there is growing evidence that blacks have lower levels of subclinical coronary artery disease than whites, despite the presence of more atherosclerotic risk factors. The prevalence and incidence of coronary heart disease reportedly increases with the increasing level of \textit{CorCP}. Strikingly lower
levels of CorCP, as well as calcified plaque in the carotid arteries and infrarenal abdominal aorta of diabetic black men, were detected compared with white men, despite increased carotid artery IMT and more severe nonlipid CVD risk factors.46 Black men in DHS families had a median CorCP of 166 (mean±SD, 1233±2583) compared with a median of 1336 (mean±SD, 2988±4355) in white men, despite a greater percentage of diabetic family members, more current smokers, higher hemoglobin A1c, blood pressure, albuminuria, and low-density lipoprotein cholesterol in blacks. In contrast, black men had higher HDL and lower triglyceride levels than white men. These results are consistent with reports from the Multi-Ethnic Study of Atherosclerosis (MESA).47 diabetic MESA participants,48 and a report by Budoff et al.49 The Dallas Heart Study did not detect ethnic differences in CorCP; however, black subjects had far more severe nonlipid CVD risk factors than whites without more severe CorCP.50

These reports demonstrate consistent ethnic differences in CorCP in response to conventional CVD risk factors. Albuminuria, more often observed in diabetic blacks, might be expected to increase CorCP, the opposite of what is observed.51 Blacks ingest less dietary calcium than do whites, which could contribute to the observed ethnic differences in CorCP.52 Blacks also have increased bone mass, reduced rates of osteoporosis and bone fracture, and manifest skeletal resistance to the effects of parathyroid hormone, relative to whites.53 Because inverse relationships have been observed between vascular calcification and bone mineral density,54,55 it is possible that common mechanisms involved in calcium metabolism underlie the propensity for whites to develop CorCP and osteoporosis, relative to blacks. Previous examination of common agents in these processes have not been conclusive, because results from the few epidemiological studies examining the contributions of calcium-bone metabolism, vitamin D levels, and parathyroid hormone to the variance in CorCP have evaluated relatively small numbers of black participants.56–59 These studies suggest that with equal access to health care, diabetic and hypertensive blacks are at enhanced risk (compared with whites) for developing small vessel intrarenal and intracranial vascular disease and lower risk for large vessel coronary, carotid, and renal artery disease. It is likely that biological factors, as well as environmental factors (behavioral, dietary, and exposure histories) contribute to these disease patterns.

Genetic Factors in Diabetic CVD

Many genes and polymorphic sites have been examined for their role in modifying CVD risk. Polymorphisms in the calpain 10 (CAPN10), protein tyrosine phosphatase 1B (PTP1B), peroxisome proliferator-activated receptor γ (PPARG), hepatic nuclear factor 4α (HNF-4α), insulin receptor (INSR), and insulin receptor substrate (IRS1, IRS2) genes, to name but a few, have been associated with risk for type 2 DM. It is intuitive that individuals with equal severity and duration of type 2 DM would be prone to different degrees of complications based on their underlying genetic susceptibility. This concept is clearly illustrated by the analysis of diabetes and CVD-associated PTP1B and CAPN10 gene polymorphisms. PTP1B gene polymorphisms may contribute, in part, to ethnic differences in severity of clinical coronary artery disease and subclinical CVD (ie, amount of CorCP). The Pro12Ala PPARγ polymorphism has been associated with protection from coronary heart disease60 and with reduced carotid artery IMT.61

CAPN10 on chromosome 2q37 encodes a nonlysosomal cysteine protease and has been associated with susceptibility to type 2 DM in multiple populations. Goodarzi et al62 recently detected association between haplotypes in CAPN10 and both insulin sensitivity and insulin secretion in nondiabetic Mexican Americans. The CAPN10 haplotype associated previously with type 2 DM was also found to confer susceptibility to increasing carotid artery IMT. This association likely accounts for the frequent coexistence of diabetes and extracranial carotid artery disease in Hispanic Americans.

The chromosomal region 20q12-q13.1 contains a gene for maturity onset diabetes of the young (MODY1), an autosomal dominant form of early onset DM. There is also strong evidence,63 subsequently widely confirmed,64–66 that 20q12-q13.1 contains ≥1 additional gene that contributes to type 2 DM. The PTPNI gene on 20q encodes protein tyrosine phosphatase-1B (PTP1B), ubiquitously expressed and involved in the regulation of several signaling pathways. Extensive genetic analysis of PTP1B has yielded strong evidence of association with type 2 DM in white diabetic cases and controls67 and with measures of glucose homeostasis (insulin sensitivity index and fasting glucose concentrations) in Hispanic Americans.68

Associations between the PTP1B gene and subclinical CVD were explored in white members of DHS families. The same SNPs and the common haplotype that contributed to type 2 DM susceptibility and insulin resistance were significantly associated with CorCP.69 The PTP1B CorCP risk haplotype was present in 41% of white subjects, supporting the “common variant” hypothesis. In contrast, the PTP1B haplotypes demonstrated previously to be protective from type 2 DM were not associated with alterations in the amount of CorCP.

Additional pathways of interest in susceptibility to calcified atherosclerotic plaque include inhibitors of mineralization (ie, Fetuin-A, matrix gla protein, osteopontin, osteoprotegerin, and bone morphogenetic protein 7) and promoters of vascular mineralization (bone morphogenetic protein 2, phosphorus, and vitamin D). Fetuin-A is an important inhibitor of tissue calcification. Polymorphisms in the corresponding α2-Heremans-Schmid Glycoprotein (AHSG) gene are associated with serum fetuin-A concentration, free phosphate concentration, and death from CVD.70,71 Fetuin-A protein levels are reduced in individuals with kidney failure and are associated with an increased risk of calcified vascular plaque, inflammation, CVD, and all-cause mortality.72,73 The level of calcified atherosclerotic plaque in an individual may vary based on AHSG gene variation. Multiple SNPs and 2 haplotype blocks encompassing the exon 6 to exon 7 region of the AHSG gene were associated with CorCP in white subjects with type 2 DM.74 These data strongly implicate AHSG and fetuin-A in the development of calcified atherosclerotic plaque in individuals with type 2 DM.

An important concept in understanding the role of PTP1B polymorphisms in type 2 DM and subclinical CVD is the lack
of linkage of chromosome 20q markers to type 2 DM in black families.\textsuperscript{75} In addition, \textit{PTP1B} gene polymorphisms were excluded from involvement in diabetes susceptibility in blacks.\textsuperscript{67} Thus, the \textit{PTP1B} gene association in DM seems to be ethnic specific. Different profiles of genes and variants that contribute to DM by population are likely to contribute to observed ethnic differences in target organ complications. For example, the lower amounts of CorCP and clinical CVD among diabetic blacks may reflect the lack of \textit{PTP1B} associations with disease. Other CVD-associated genes, either diabetes related or unrelated (ie, \textit{AHSG}), are likely to contribute to individual and ethnic-specific susceptibility to the vascular complications of DM.

\section*{Perspectives}

Diabetes and its complications result from complex interactions between environmental and inherited factors. Familial aggregation and ethnically diverse patterns of type 2 diabetes-associated renal and cardiovascular complications likely reflect, in part, genetic susceptibility. It would be expected that conventional risk factors for diabetic CVD and renal disease, as well as therapies for hyperglycemia, will have differential impacts on disease progression based on host genetic factors. Several genomic regions underlying nephropathy susceptibility seem to be shared between racial groups, whereas others may be limited to certain ethnic groups. The impact of \textit{PTP1B} gene polymorphisms in diabetic CVD may contribute to the increased coronary artery disease risk observed in the white population. Pharmacogenomic differences exist in response to congestive heart failure therapy based on ethnicity.\textsuperscript{76} It is also likely that underlying ethnic differences in vascular disease susceptibility, including the propensity to develop small vessel renal and intracranial arterial disease in blacks and large vessel extracranial carotid artery, coronary artery, and main renal artery atherosclerosis in whites reflect genomic differences between ethnic groups.

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None.

\section*{References}


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