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Protein Kinase C- β Gene Variants and Type 2 Diabetes–Associated Kidney Failure: What Can We Learn From Gene Association Studies in Diabetic Nephropathy?

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The paradigm that glucotoxicity alone mediates the risk for kidney failure and cardiovascular events in individuals with type 2 diabetes has given way to a complex interplay between hyperglycemia (permissive environment) and inherited predisposition leading to a higher likelihood of complications. Improving glycemic, lipid and hypertension control reduces diabetes-associated micro- and macrovascular disease. Nonetheless, inherited susceptibility to kidney failure due to type 2 diabetes clearly exists. Nephropathy clusters more tightly in multiply affected families with type 2 diabetes than does diabetes *per se* and familial aggregation of kidney failure, albuminuria, glomerular filtration rate (GFR) and renal histology are widely observed and independent from hyperglycemia.^{1–3}

The search for genetic susceptibility in both type 1 diabetes and type 2 diabetes -associated kidney failure began before the human genome was sequenced or its haplotype block structure appreciated. Analyses progressed from underpowered candidate gene studies using one or two polymorphic markers failing to capture all variation in genes, to the current era of unbiased genome-wide approaches including family-based linkage scans, genome-wide association studies (GWAS) employing one million or more markers (single-nucleotide polymorphisms; SNPs), and mapping by admixture linkage disequilibrium (MALD). Each approach has theoretical advantages: linkage scans may detect uncommon variants with strong effects in select families, GWAS can identify common variants with relatively modest effect, and MALD identifies genes in admixed populations where ancestral groups have differential disease risk. Together, these approaches have the potential to identify new mechanisms, treatment strategies and screening options for patients with type 2 diabetes at risk for diabetic nephropathy.

WHAT DOES THIS IMPORTANT STUDY SHOW?

The worldwide epidemic of type 2 diabetes has not spared Asia. Employing a straightforward case-control design and investigating a candidate gene previously implicated in vascular and renal complications of diabetes,^{4;5} Ma and colleagues detected evidence of association between the protein kinase C- β gene (PRKCB1; gene symbol, *PRKCB*) and kidney failure in type 2 diabetes in Chinese patients.

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The study cohort included 1338 unrelated patients with type 2 diabetes from the Hong Kong Diabetes Registry enrolled over 3 years with an initial GFR > 15 ml/min/1.73m².⁶ Uniform renal phenotypic data were automatically downloaded to a central database over the ensuing 8 years whenever clinic visits occurred. Cases with kidney failure were prospectively captured, not only those requiring dialysis, but also uremia-related deaths and chronic kidney disease (CKD) stage 5 in non-dialyzed patients. In contrast to recruiting participants from dialysis units, the potential to detect “survival” genes enriched in those living to initiate dialysis was minimized by the prospective design.⁶

Dialysis-dependent kidney failure was selected as an unambiguous primary phenotype. Intermediate phenotypes including albuminuria and rate of change in GFR have been examined in GWAS.⁷ However, diabetic nephropathy does not uniformly progress from hyperfiltration with microalbuminuria to increasing proteinuria with falling GFR. Some patients develop progressive reductions in GFR without heavy proteinuria, limiting the value of intermediate phenotypes.⁸ Genes associated with kidney function and albuminuria have been identified in population-based cohorts that were not enriched for advanced nephropathy. As a result, many genes associated with change in GFR are not associated with development of advanced kidney failure. In addition to the value of a kidney failure phenotype, this report replicated the PRKCB1 association in a validation cohort with earlier stages of CKD from the same Registry.⁶

The authors evaluated 18 common SNPs spanning regions upstream and downstream from PRKCB1. Tested SNPs had low recombination fractions, 12 were tag-SNPs providing information on haplotype blocks (linked markers inherited together due to strong linkage disequilibrium), and 6 were chosen due to prior evidence of association. Thus, an intensive evaluation across the PRKCB1 gene was performed.

Two PRKCB1 SNPs, rs3760106 and rs2575390, were significantly associated with kidney failure with type 2 diabetes in the study cohort and with earlier stages of CKD in type 2 diabetes in the validation cohort.⁶ These SNPs implicate a single genomic region, as they were often co-inherited. An important observation was the strength of disease association; odds ratios (OR) of 2.25–2.26 were detected.

HOW DOES THIS STUDY COMPARE WITH PRIOR STUDIES?

More than two decades of candidate gene association studies have yielded few replicated genes with major effects in kidney failure with type 2 diabetes. Box 1 summarizes the main gene associations reported in diabetic nephropathy. For example, the angiotensin I-converting enzyme gene (*ACE*) locus is widely touted as a major diabetic nephropathy susceptibility locus.⁹ *ACE* exhibits variable association with diabetic kidney disease, stronger effects are seen in Asians and patients with type 1 diabetes. Although PRKCB1 has a functional rationale to be a candidate gene for analysis, the pathophysiology of diabetic nephropathy remains poorly understood and it is difficult to correctly choose causative genes based on underlying biology. This report by Ma et al⁶ adds to the overall portrait of diabetic nephropathy, providing insights in the Asian population for which few reports exist. While unbiased scans across the genome appear to have the greatest chance for success, this study demonstrates there is room for multiple investigative approaches.

Box 1

Potential susceptibility genes for diabetic nephropathy

- *ACACB*: acetyl-coenzyme A carboxylase β
- *ACE*: angiotensin I-converting enzyme

- *CARS*: cysteinyl-tRNA synthetase
- *CNDP1*: carnosinase 1
- *ELMO1*: engulfment and cell motility 1
- *NOS3*: endothelial nitric oxide synthase (eNOS)
- *EPO*: erythropoietin
- *FRMD3*: FERM (4.1 protein ezrin, radixin, moesin) domain containing 3
- *GREM1*: gremlin 1
- *NCALD*: neurocalcin δ
- *PVT1*: plasmacytoma variant translocation
- *PRKCB*: protein kinase C- β 1 (PKC- β 1)
- *SOD1*:superoxide dismutase 1

In contrast to candidate gene association studies, GWAS in type 2 diabetes have detected more than 40 associated genes.¹⁰ Type 2 diabetes genes generally have weaker effects on disease risk (e.g., OR ≤ 1.4), relative to *PRKCB1* and other recently detected nephropathy susceptibility genes. An OR of 1.4 reveals a 40% increased chance of disease for each risk allele inherited. *PRKCB1* nephropathy variants had stronger effects on kidney failure in type 2 diabetes risk with OR ~ 2.25 (95% confidence interval ranged from 1.31 to 3.88 given the sample size). This effect in diabetic nephropathy is similar to that seen with the acetyl co-enzyme A carboxylase β (*ACACB*; OR, 1.6–2.4) and engulfment and cell motility 1 (*ELMO1*; OR, 1.24–2.7) genes^{11–13} and is stronger than for the genes encoding FERM domain-containing 3 protein (*FRMD3*; OR, 1.45) and cysteinyl-tRNA synthetase (*CARS*; OR, 1.36) genes.¹⁴ In contrast, the apolipoprotein L1 gene (*APOLI*) is far more strongly associated with non-diabetic nephropathy in African Americans (OR of 10.5 in focal segmental glomerulosclerosis and 7.3 in kidney failure attributed to hypertension; $p=10^{-63}$), exhibiting among the strongest association in any complex human disease.¹⁵ Based on initial GWAS results, a kidney failure in type 2 diabetes susceptibility gene with an effect size similar to that of *APOLI* is unlikely to exist.

WHAT SHOULD CLINICIANS AND RESEARCHERS DO?

The protein kinase C (PKC) pathway has been intensively studied in patients with diabetes. Retinal, renal, neuronal, and cardiovascular complications have been attributed to different PKC- α , - β , - δ , and - ϵ isoforms.¹⁶ The lipid second messenger diacylglycerol (DAG) activates this pathway and hyperglycemia increases intermediate product synthesis by the glycolytic pathway, further increasing synthesis of DAG.¹⁷ The renal consequences of an activated DAG-PKC pathway can include increased endothelial cell permeability to albumin, mesangial expansion, enhanced production of profibrotic molecules (transforming growth factor [TGF]- β 1, connective tissue growth factor [CTGF]), and proximal tubular cell fibronectin synthesis.^{18;19} Knocking-out PKC- α or PKC- β in diabetic mice reduces renal production of collagens type III/IV and fibronectin and prevents loss of nephrin and Wilms tumor 1 (WT-1) protein from podocytes.^{20;21} These findings implicate variation in the *PRKCB1* gene with diabetic nephropathy and suggest that more detailed appraisals of this gene and genes in common pathways are warranted.

PKC- β has 2 subtypes (PKC- β I and PKC- β II) generated by alternative splicing of the PKC- β gene. These subtypes have distinct subcellular locations and functions, often with opposing

effects.²² For example, PKC- β I is pro-apoptotic, while PKC- β II is anti-apoptotic.²³ Insulin appears to regulate alternative splicing in a dose-dependent fashion by switching the subtype expressed in myocytes from PKC- β I to PKC- β II. Transfection-induced overexpression of PKC- β II in fibroblasts significantly enhances insulin-induced glucose transport, results not observed with overexpression of PKC- β I.²⁴ Hence, nephropathy-associated PRKCB1 polymorphisms may lead to progressive kidney failure in type 2 diabetes by effects on alternative splicing, changes in promoter activity or altered binding of transcription factors.⁶

The recent genetics literature reveals an important role for dysregulated lipid metabolism in the pathogenesis of diabetic (and non-diabetic) nephropathy, a previously underappreciated area of investigation. An *ACACB* variant confers risk for diabetic nephropathy,^{11;12} *APOLI* gene variants predispose to glomerulosclerosis in non-diabetic African Americans,¹⁵ the lipid mediator DAG regulates the important PKC pathway, and the enzyme encoded by *ACACB* plays a pivotal role in fatty acid oxidation. Fatty acid accumulation in cells and tissues (along with their esters, including DAG) impairs insulin signaling, reduces glucose tolerance and leads to type 2 diabetes (lipotoxicity).²⁵ High tissue levels of DAG, potentially accumulating due to reduced fatty acid oxidation or elevated circulating fatty acid levels, represents another potential route of PKC- β activation in patients with type 2 diabetes.^{26;27}

An integrated model linking abnormal fatty acid metabolism with PKC pathway defects can be envisioned for diabetic nephropathy. Initiation and progression of diabetic nephropathy likely result from inheriting variants in multiple genes. Diabetic patients with *ACACB*-mediated alterations in fatty acid metabolism may develop proteinuria and synergistic activation of the PKC pathway could confer additional risk.²⁸ Diabetic hyperfiltration and microalbuminuria may be influenced by *ACE* and endothelial nitric oxide synthase (eNOS) polymorphisms with altered glomerular vascular tone, production of reactive oxygen species and advanced glycation end-products. Harboring additional mutations in pathways that regulate extracellular matrix production and/or apoptosis (*e.g.*, in the genes for PKC- β 1, carnosinase 1 [CNDP1], ELMO1) could augment downstream effects leading to advanced kidney failure. Analyses incorporating these variants may prove enlightening for developing genetic risk profiles of diabetic nephropathy susceptibility.

In conclusion, this study demonstrates that a properly performed and adequately powered candidate gene association analysis, in addition to complex GWAS, linkage and MALD approaches, can detect novel loci with increased susceptibility to kidney failure in type 2 diabetes. Remaining questions include the effect of PRKCB1 variation on susceptibility to type 1 diabetes-associated nephropathy and in non-Asian populations, as well as whether interactions exist between the major diabetic nephropathy susceptibility genes that have been detected to date. Medications affecting fatty acid oxidation may potentially benefit diabetic individuals with risk variants in the PRKCB1 gene and *ACACB*. Future pharmacogenomic analyses will inform us whether individuals with PRKCB1 risk variants respond differently to blood sugar lowering and existing renoprotective therapies. Diabetic nephropathy genes and their associated pathways will provide novel screening tools and therapeutic options to treat patients with diabetes at risk for kidney failure.

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