The Challenging Search for Diabetic Nephropathy Genes

Donald W. Bowden[1] and Barry I. Freedman[2]

It is widely appreciated that macro- and microvascular complications, rather than hyperglycemia per se, are major contributors to morbidity and mortality in diabetes. In this issue of *Diabetes*, Williams et al. (1) report the results of a meta-analysis of genetic data from three moderately sized studies of patients with type 1 diabetes (T1D) and nephropathy. This report illustrates several challenges inherent in the genetic analysis of diabetes complications and is another step toward understanding the genetic basis of risk for diabetic nephropathy (DN). Insights into the genetics of DN will potentially lead to improved prediction of DN and novel approaches to prevent this serious complication of diabetes.

There is compelling evidence in support of a major genetic component for diabetes complications, especially DN. Efforts to identify genes contributing to T1D and type 2 diabetes (T2D) have been highly successful, but with few exceptions, there is little evidence that diabetes-associated variants associate with complications. Thus, diabetes complications appear to have an independent genetic basis. The profound public health impact of DN has motivated the performance of multiple genetic studies. However, these studies are complicated by issues of disease origin (T1D or T2D), ethnicity (European and European American, African American, Hispanic, Asian), competing cardiovascular risk, and variable diagnostic criteria (glomerular filtration rate and albuminuria in mildly affected individuals, variable severity of chronic kidney disease or end-stage renal disease [ESRD] requiring renal replacement therapy). Consequently, existing genetic association studies of DN have included a mosaic of diabetes type, ethnic groups, and phenotypes. These methodological dissimilarities are complicated further by different technical approaches ranging from targeted studies of individual genes to various platforms for genome-wide analysis.

Williams et al. (1) combined genetic data from three T1D cohorts: the U.K.-R.O.I., FinnDiane, and U.S. GoKinD studies, all of which contain participants of European ancestry. Important strengths of these combined studies include large (for DN studies) sample sizes of 3,162 T1D nephropathy (T1DN) case subjects and 3,845 control subjects, as well as focused efforts to harmonize phenotypes across studies. Genes with evidence of DN association from several recent studies and loci associated in an informatics-based meta-analysis of published DN genetics studies were targeted (2). With this approach, the authors note that they had sufficient power to detect evidence of association with *P* values in the range of 0.001. This research design (targeting specific genes) circumvents the power issues that make it difficult to identify significant associations using genome-wide approaches. In addition to evaluating specific single nucleotide polymorphisms (SNPs) from the primary literature, in some cases, such as the *ELMO1* gene, the investigators also performed "locus-wide" analysis to assess whether other SNPs near the index SNP were associated. Each approach was pursued with rigorous testing for statistical significance. Unfortunately, compelling evidence of association was not observed for any loci. One possible exception was a promoter variation in the erythropoietin gene (*EPO*). A single SNP was strongly associated with T1DN (combined with proliferative retinopathy) in a prior report that included the GoKinD samples (3). While evidence of association was not observed in U.K.-R.O.I. or FinnDiane, the *P* value for association remained strong in a combined analysis with GoKinD. Consistent with their rigorous standards, the authors felt this reflected limited evidence of association. A similar outcome was observed with other SNPs such as in *FRMD3:* strong association in U.S. GoKinD, but no association in other samples.

Working primarily from the results of the meta-analysis of Mooyaart et al. (2) may have introduced limitations. Mooyaart et al. evaluated a diverse set of studies with varying participant numbers, ethnicities, and disease definitions. This approach may minimize evidence of association in a specific ethnic group or genes associated with a discrete phenotype. In addition, several recent manuscripts identified additional loci in better powered genome-wide association studies (GWASs) of diabetic ESRD in African Americans (4) and quantitative measures of kidney function in European-derived samples (5). It will be interesting to see whether variants from these reports are associated in this T1DN sample.

Another challenge of the study presented by Williams et al. is that it addresses only one element in the mosaic of DN: T1DN in European-derived populations defined by ESRD. Realistically, this is the only practical approach, but this study consequently reflects most directly on variants that have been identified using this study design. As noted, researchers have investigated DN in various populations and with differing definitions of DN (e.g., albuminuria, chronic kidney disease, or ESRD). For example, the association between *ELMO1* was initially observed in a GWAS of Japanese T2D-associated nephropathy (T2DN) (6) and, as the authors note, followed up in a study of African American T2DN (7) and then European American T1DN (8), the latter study in GoKinD itself. These contrasts are representative of the many issues that remain unresolved in DN genetics. In a background of largely negative comparisons, there are hints that some variants are associated with

From the [1]Department of Biochemistry, Centers for Diabetes Research and Genomics and Personalized Medicine Research, Wake Forest School of Medicine, Wake Forest University, Winston-Salem, North Carolina; and the [2]Department of Internal Medicine, Section on Nephrology, Wake Forest School of Medicine, Wake Forest University, Winston-Salem, North Carolina.

Corresponding author: Donald W. Bowden, dbowden@wfubmc.edu.

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nephropathy across ethnicities and disease classifications (T1DN or T2DN), *ELMO1* being a good example. However, these observations were countered by this well-powered study. It must be emphasized that renal failure genes may be risk factors only in specific ethnic groups. An obvious example is the powerful association of apolipoprotein L1 gene (*APOL1*) variants with nondiabetic ESRD in African Americans (9–11). The likely causative variants are virtually absent in European-derived populations. Similarly, although there is some evidence that the T1DN-associated *FRMD3* (identified in European-derived samples) is associated with T2DN in African Americans (12,13), there is no compelling evidence that the genes for T1DN and T2DN will necessarily be the same even in the same ethnic group.

In our opinion, the greatest limitation of genetic research in DN, regardless of disease origin or population group, is the limited number of appropriate samples for analysis. The meta-analysis reported by Williams et al. (1) is an important step toward addressing this barrier, but the total sample of 3,162 case subjects and 3,845 control subjects likely lacks sufficient power to detect novel loci in a GWAS analysis without including large numbers of additional participants or additional cohorts. Compared with genetic studies of diabetes, the numbers available for DN research are modest, and strict phenotypic criteria, as used in this study, though perhaps ideal, make it difficult to increase sample size. In some ethnicities, such as African Americans, alternative approaches are required because of the very high prevalence of renal involvement in T2D.

In summary, this work represents an important step in DN in which large, better powered, and more comprehensive genetic studies will begin to reveal the inherited contributions to this devastating diabetes complication. This study is an example of what will be needed to move the field forward: combined analysis of well characterized datasets. Fortunately, multiple investigative groups are committed to these goals, and the future should see larger and increasingly informative genetic studies in DN.

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