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The Genetic Risk of Kidney Disease in Type 2 Diabetes

Marcus G. Pezzolesi, PhD, MPH^{1,2} and Andrzej S. Krolewski, MD, PhD^{1,2}

¹Section on Genetics and Epidemiology, Research Division, Joslin Diabetes Center, Boston, MA 02215

²Department of Medicine, Harvard Medical School, Boston, MA 02215

Abstract

Evidence of familial aggregation of diabetic nephropathy (DN) in type 2 diabetes and the heritability of its related traits provide compelling evidence that genetic factors contribute to their susceptibility. Segregation analyses suggest the existence of at least one major DN susceptibility gene as well as multiple other genetic factors with small to moderate effects on its risk. For more than 20 years, these studies have motivated investigators working to identify the causal genes responsible for the development of DN. During this period, advances in genomics and evolving technology have improved our understanding of the genetic basis of DN and revolutionized our ability to identify genes that underlie this disease. In this review, we discuss the major approaches being used to identify DN susceptibility genes in T2D and highlight the salient findings from studies where these approaches have been implemented. The recent advent of next-generation sequencing technology is beginning to impact DN gene mapping strategies. As the field moves forward, family-based approaches should greatly facilitate efforts to identify variants in genes that have a major affect on the risk of DN in T2D. To be successful, the ascertainment and comprehensive study of families with multiple affected members is critical.

Introduction

Diabetic nephropathy (DN) is a major late complication of diabetes that affects approximately 40% of all patients with diabetes and remains the leading cause of end-stage renal disease (ESRD) in the United States.^{1–3} As the incidence of type 2 diabetes (T2D) continues to rise in the United States, and across the globe, so to are the personal and societal burden's associated with this complication.

Investigations on the familial clustering of DN in T2D and the heritability of DN and its related traits provide compelling evidence that genetic factors contribute to its susceptibility and have motivated studies aimed at identifying the causal genes responsible for its development. For more than 20 years, investigators have been working to identify the genes that underlie its susceptibility. During this period, advances in genomics have expanded our understanding of genetic variation across our genome and its contribution to disease and facilitated cutting-edge technologies that have revolutionized our ability to identify the genes that underlie these conditions.

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Corresponding author: Marcus G. Pezzolesi PhD, MPH, Section on Genetics and Epidemiology, Rm. 445C, Joslin Diabetes Center, One Joslin Place, Boston, MA 02215, marcus.pezzolesi@joslin.harvard.edu.

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In this review, we discuss the approaches used to identify DN susceptibility genes in T2D, including their key findings, and present our perspective on future studies in this field.

Familial Clustering of Nephropathy and Estimates of its Heritability in T2D

Evidence of familial aggregation supports the notion that genetic factors play a major role in the susceptibility of DN in T2D.^{4–7} In the earliest investigation of familial clustering of DN among families with T2D, Pettitt et al. examined the risk of proteinuria among 316 Pima Indian families with diabetes in two generations. In this study, the risk of proteinuria among diabetic offspring with a parent with proteinuria was 1.8 times higher than that of offspring of diabetic parents without proteinuria.⁴ The adjusted prevalence of proteinuria among individuals with one diabetic parent with proteinuria was 23%; compared to only 14% among offspring with two diabetic parents with normoalbuminuria. The prevalence of proteinuria among offspring with two diabetic parents with proteinuria was even greater, with 46% of these individuals having this complication.

In 52 multi-generational African-American families, Freedman et al. found that 37% of T2D-induced ESRD patients had either first-, second-, or third-degree relative with ESRD, compared to only 7% of T2D controls.⁵ Diabetic individuals from these families with a relative with ESRD were at an eight-fold increased risk of developing ESRD. Studies by Faronato et al. and Canani et al. similarly demonstrated that T2D siblings of probands with DN from Caucasian families had 3 to 4 times the risk of developing micro- and macroalbuminuria compared to sibling of normoalbuminuric probands.^{6,7} Faronato et al. also confirmed a previous report by Gruden et al. that demonstrated that albumin excretion rate (AER) was increased in non-diabetic family members of T2D patients.^{6,8}

To more precisely determine the relative contribution of genetic factors to DN in T2D, we and others have estimated the heritability (h^2 ; i.e., the proportion of total variation of a trait due to genetic effects) of its correlated traits (i.e., urinary albumin excretion rate (AER) and estimated glomerular filtration rate (eGFR)) in families with T2D.^{9–13} In 96 large, multi-generational families that included 630 individuals with T2D and 639 individuals with normoglycemia enrolled in the Joslin Study on the Genetics of Type 2 Diabetes, Fogarty et al. estimated that 27% of the variance in albumin-to-creatinine ratio (ACR) was genetically determined among all family members, irrespective of their diabetes status.⁹ In analyses restricted to diabetic individuals, this estimate rose slightly to 31% and, supporting previous reports of familial clustering of AER among non-diabetic family members, h^2 was estimated to be 0.20 in non-diabetics individuals from this collection.

A subsequent analysis of the Joslin Study on the Genetics of Type 2 Diabetes collection restricted to families with a middle-age at onset of T2D reported similar estimates of heritability with ACR, ranging from 0.20 in all family members to 0.39 in relative without diabetes.¹¹ An important strength of the Joslin T2D family collection is that its members were ascertained for studies on the genetics of T2D, not kidney complications. As such, these estimates of heritability are unlikely to be biased due to an enrichment of DN cases. Reinforcing the estimates obtained from this collection, Forsholm et al. and Langeled et al. reported similar heritability for AER in 267 nuclear T2D families from Finland ($h^2 = 0.30$) and for ACR in 310 T2D sibling pairs from the United States ($h^2 = 0.46$ in T2D members and $h^2 = 0.35$ in all family members).^{10,12}

To evaluate the possible mode of inheritance of ACR in families with T2D, we performed a formal quantitative segregation analysis of this trait in members of the Joslin Study on the Genetics of Type 2 Diabetes collection.¹⁴ In this analysis, evidence for the genetic effects on ACR was derived from its transmission between relatives across large pedigrees; an approach that provides substantial power in assessing this effect over studies limited to

nuclear families. In models where the genetic effect was assessed separately in all member and in T2D members alone, the model that most completely described the control of ACR levels in these pedigrees combined the effects of at least one major locus (with a relatively common allele frequency between 0.25 to 0.40) with significant residual genetic variation that could be due to multiple other genetic factors. These results are consistent with a previous segregation analysis of overt nephropathy by Imperatore et al. that also supported the existence of a major DN gene with a common allele frequency in a collection of 715 nuclear Pima Indian families.¹⁵

eGFR has also been shown to be a significantly heritable trait in families with T2D.^{12,13} The first study to investigate this estimated the heritability of eGFR to be 0.75 among Caucasian T2D sibling pairs and 0.69 in analyses that included all available family members.¹² Similarly, we found eGFR to be highly heritable T2D subjects (h^2 ranging from 0.29 to 0.47) and all family members (h^2 ranging from 0.28 to 0.31) from the Joslin Study on the Genetics of Type 2 Diabetes collection.¹³ To date, no formal segregation analysis of eGFR in T2D has been published.

Strong evidence of familial aggregation and the heritability of DN in T2D provide compelling evidence that DN and its related traits are influenced by genetic factors and suggest a complex, multifactorial mode of inheritance with one or more major susceptibility genes. While a shared environment might contribute to some of the familial clustering of renal disease in T2D, these studies support the hypothesis that the increased risk DN in T2D is partly due to a shared gene or set of genes among affected family members. Together, these data have motivated investigations aimed at identifying the specific chromosomal regions that harbor genes contributing to its susceptibility. In the next two sections, we discuss the major approaches that are currently being used to identify DN susceptibility genes and highlight the salient findings from studies where they have been implemented.

Genome-wide Linkage Analyses Identify Several Susceptibility Loci for DN in T2D

The major efforts to identify genes that cause DN in T2D have come from family-based genome-wide linkage studies. These studies entail genotyping polymorphic genetic markers in families, typically either small nuclear families or multigenerational extended families, and evaluating the correlation between the phenotype of interest and the pattern of inheritance of these markers. Earlier studies used a set of ~300 to 400 highly polymorphic microsatellite markers dispersed across the entire genome. Conventional studies now use dense linkage panels comprised of 5,000 or more single nucleotide polymorphisms (SNPs). An advantage of linkage-based approaches is that they offer a model-free screen of markers across the genome; however, linkage studies are limited with respect to the magnitude of the underlying genetic effect and the resolution with which they are able to pinpoint susceptibility loci. Linkage studies are generally powered to identify major disease loci (i.e., those with effect sizes greater than 2.0) and typically localize linkage signals to regions several mega-basepair in length.

To date, a total of eleven complete genome-wide linkage scans have been published for DN in T2D.^{11,13,15–23} The major findings from these studies are summarized in Tables 1 and 2. While several distinct regions across the genome provide some evidence of linkage, consistent linkage with DN and its related phenotypes has been localized to a number of potential candidate loci.

The strongest evidence of linkage with DN was identified on chromosome 18q (maximum LOD score (MLS) = 6.1) in 18 extended Turkish families with 115 members with T2D.¹⁶

Evidence of linkage to this same region was shown in both African-American T2D patients with ESRD¹⁷ and with eGFR in 378 multi-ethnic families from the Family Investigation of Nephropathy and Diabetes (FIND) collection.²³ In the FIND study, the findings at this locus were primarily driven by Mexican-American families who comprised 52% of the families enrolled in this collection. Resequencing efforts in 135 T2D DN cases and 107 T2D non-DN controls later identified a significant association at a trinucleotide repeat in exon 2 of the carnosinase 1 gene (*CNDP1*) that encodes a variable stretch of leucine residues (five, six, or seven leucines).²⁴ In this study, the five-leucine allele was present in only 59% of chromosomes in patients with DN compared to 88% in those without DN. Functional studies confirmed the protective effects of this allele by demonstrating its ability to inhibit the production of extracellular matrix components in cultured human podocytes exposed to high glucose. Similarly, transforming growth factor-beta production was reduced in cultured mesangial cells exposed to high glucose. Additional support of this polymorphism's role in DN was provided by its association in 858 European-American subjects (294 ESRD patients with T2D, 258 T2D controls, and 306 healthy controls).²⁵

A second locus with consistent support of linkage to DN in T2D localizes to chromosome 3q. Bowden et al. provided significant evidence of linkage to this region with early-onset ESRD in an optimum subset analysis of 48 African-American families.¹⁷ We reported suggestive evidence of linkage with eGFR near this same region in a genome-wide linkage scan performed in 63 multigenerational European-American families from the Joslin Study on the Genetics of Type 2 Diabetes collection.¹³ Linkage has also been reported on chromosome 3q in separate studies of sibling pairs concordant for type 1 diabetes (T1D) but discordant for DN from the Joslin Study of Genetics in Type 1 Diabetes collection and Finland.^{26,27} As part of a targeted linkage study of candidate loci performed in 66 sibling pairs, we identified a strong linkage signal approximately 15 kilo-basepair downstream from the angiotensin II type 1 receptor gene (*AGTR1*).²⁷ A genome-wide linkage scan of 83 Finnish sibling pairs reported evidence of linkage at this same region.²⁶ Although the candidate gene underlying these signals has yet to be defined, these studies, as well as a recent effort to fine-map this region in unrelated T1D cases and controls²⁸, reinforce the likelihood that this region harbors a gene (or genes) that contribute to the risk of DN and suggests that this susceptibility locus may be common to both T1D and T2D.

Evidence of linkage with DN phenotypes is also mounting at loci on chromosomes 7p and 22q.^{11,13,17,22} On chromosome 7p, significant linkage (MLS = 3.6) was first reported in an ordered subset analysis of African-American families with ESRD patients and a long duration of T2D.¹⁷ In a study by Placha et al., a linkage scan for genes controlling variation in eGFR in 406 T2D and 428 non-diabetic members from 63 extended families in the Joslin Study on the Genetics of Type 2 Diabetes collection identified strong evidence for linkage at this same region (MLS = 4.0).¹³ Most recently, suggestive evidence for linkage on chromosome 7p (MLS = 2.81) has also been reported in an expanded linkage scan of the FIND collection that now includes 1,235 multi-ethnic T2D families.²² In the Joslin collection, a second scan for regions linked with variation in urinary albumin excretion found significant linkage on chromosome 22q (MLS = 3.7).¹¹ Support for this region has also recently been confirmed in Mexican-American families from the FIND collection.²²

On chromosome 22q, the non-muscle myosin heavy chain 9 gene (*MYH9*), expressed in both glomerular podocytes and mesangial cells, represents a particularly interesting candidate gene.^{29,30} Genetic variation at the *MYH9* locus is strongly associated with non-diabetic nephropathy, including focal segmental glomerulosclerosis, hypertensive nephropathy, and non-diabetes associated ESRD.^{31–33} As first demonstrated by Freedman et al., *MYH9* SNPs also appear to contribute to the risk of nephropathy in African-American T2D patients.³³ In this study, a comparison of 751 ESRD patients with clinically-diagnosed

T2D and 227 T2D controls identified significant associations at 3 *MYH9* SNPs (rs4821480, rs2032487, and rs4821481). In a subsequent study, these same variants trended toward an association with ESRD in 536 T2D cases and 467 T2D controls of European-American ancestry.³⁴ These observations, however, were not confirmed in a recent study of T2D patients from the United Kingdom.³⁵ An important distinction between the study by McKnight et al. and the two previous reports is that the former examined these associations in T2D nephropathy patients with CKD; less than 100 of whom had ESRD. In consideration of this fact, and the support garnered from multiple linkage studies of DN in T2D, continued investigation of the role of variants in the *MYH9* region in T2D-associated DN is warranted.

Genome-wide Association Scans for DN Genes in T2D

Overall, linkage-based approaches have been quite successful in mapping loci that contribute to the risk of DN in T2D. Because of the limited resolution of this approach, however, efforts to definitively identify the genes underlying these signals continue to be challenging. Driven both by the limitations of linkage-based approaches and advances in genotyping technology, genome-wide association scans (GWASs) have generated a great deal of optimism among researchers working to identify susceptibility genes for DN. This approach to mapping genes use commercially available genotyping arrays to readily interrogate millions of common SNPs, with minor allele frequencies (MAFs) generally ranging from 5.0% to 45%, across the entire genome; a feature that offers significantly improved resolution compared to linkage studies. The underlying hypothesis of GWASs is that common variants increase susceptibility to common disease.

GWASs have proven to be extremely powerful in detecting disease loci that are associated with many complex human traits and diseases, including coronary heart disease, T1D, T2D, bipolar disorder, Crohn's disease, and rheumatoid arthritis.³⁶ Over the past several years, such studies have become an increasingly attractive approach to identify DN susceptibility genes. To date, three formal GWASs have been conducted in T2D patients with DN (Table 3).^{37–39}

The first such report was the gene-based analysis of 81,315 SNPs in 188 Japanese patients with T2D (94 cases with either proteinuria or ESRD and 94 normoalbuminuric controls).³⁹ From this discovery panel, 1,615 SNPs were selected for replication in a larger collection that included 466 T2D DN cases and 266 T2D controls. Using this two-stage approach, rs741301, located in intron 18 of the engulfment and cell motility 1 (*ELMO1*) gene on chromosome 7p, emerged as the most strongly DN-associated SNP in these collections (p -value = 8.0×10^{-6}). Subsequent functional studies by Shimazaki et al. demonstrated an increased expression of *ELMO1* in the presence of high glucose.^{39,40} Supporting its potential role in the pathogenesis of DN, *ELMO1* has also been shown to contribute to the progression of chronic glomerular injury by promoting excess TGF- β , collagen type 1, fibronectin, and integrin-linked kinase expression and dysregulation of renal extracellular matrix (ECM) metabolism.

Since the initial report by Shimazaki et al, variants at *ELMO1* have been shown to be associated with DN in multiple independent collections.^{41–43} Confirmation of *ELMO1*'s potential role in the susceptibility of DN was first demonstrated in a study by Leak et al. that identified strong associations between multiple variants located in intron 13 of *ELMO1* and ESRD in two large African-American cohorts with T2D.⁴³ Variants located in intron 13 were also associated with overt proteinuria in a family-based study of Pima Indians with T2D.⁴¹ Of note, the associations observed in Pima Indians were in the opposite direction of those observed in African-Americans. Additionally, in a comprehensive investigation of variants across this locus using GWAS data from the Genetics of Kidneys in Diabetes

(GoKinD) collections, we further established *ELMO1*'s role in conferring increased susceptibility to DN by demonstrating that *ELMO1* variants are also associated with its risk in Caucasian T1D patients.⁴² The strongest associations in this study mapped to intron 16 of *ELMO1*.

Evidence from each of these studies is consistent with *ELMO1*'s role in DN and suggests that extensive allelic heterogeneity, contributed by the diverse ancestral genetic backgrounds of the different ethnic groups examined in each of these studies, exists across this locus. We hypothesize that rare polymorphisms in *ELMO1*, either the same variants or variants in strong or complete linkage disequilibrium, may be common to each ethnic group and merely tagged by the common variants identified in each study. Further investigation of rare SNPs at the *ELMO1* locus is likely necessary to fully understand the commonality of these associations and to elucidate the mechanism(s) underlying their role in DN.

In a second GWAS aimed at identifying DN genes in T2D, Hanson et al. used a pooled genomic DNA approach to genotype 115,352 SNPs in 105 Pima Indians with T2D and ESRD and 102 Pima Indians with T2D and either normoalbuminuria or microalbuminuria.³⁸ This analysis identified strong associations at variants in the plasmacytoma variant translocation (*PVT1*) gene on chromosome 8. Subsequent fine-mapping of this locus revealed the strongest evidence for association at rs2648875 (p -value = 2.0×10^{-6}) located in intron 8 of *PVT1*. Confirmation of this association was later shown by Millis et al. in a subset of patients with ESRD from the GoKinD collections.⁴⁴

In the largest GWAS to date, a multi-stage approach that included African-American individuals with and without T2D identified several novel regions with evidence of association with T2D-associated ESRD.³⁷ As part of their approach, McDonough et al. used comparisons of T2D-ESRD cases and non-diabetic, non-nephropathy controls to identify 67 candidate SNPs that were then genotyped in T2D controls to discriminate between T2D-ESRD loci and T2D loci. In combined analyses of 1,674 T2D ESRD cases and 1,719 non-T2D controls, a total of five loci achieved p -values $< 1.0 \times 10^{-5}$. Among these, rs9493454 at AU RNA binding protein/enoyl-CoA hydratase (*AUH*) on chromosome 9 and rs7735506 at ribosomal protein S12 (*RPS12*) on chromosome 6 were highly significant in comparisons between T2D-ESRD cases and T2D non-DN controls ($n = 1,216$; p -values = 3.60×10^{-3} and 8.79×10^{-4} , respectively).

Lastly, in conjunction with their linkage analysis, Igo et al. also performed a 'sparse' genome-wide association scan of DN and ACR in the FIND collection using ~5,500 SNPs from their linkage panel.²² In this study, the strongest association with DN was observed on chromosome 18 in the American-Indian subgroup (rs1241893; p -value = 3.0×10^{-5}). On chromosome 11, associations with ACR were found at rs722317 in both European-American and Mexican-American samples (p -values = 4.6×10^{-4} and 2.6×10^{-3} , respectively; combined p -value of 7.3×10^{-5}). A complete GWAS was recently completed in this same collection that includes ~935,000 SNPs (B. Freedman, personal communication). We anticipate that this study will offer a great deal of insight to these, as well as several other loci, that contribute to DN in T2D.

The ascent of GWASs in investigations on the genetic basis of DN in T2D has shifted gene mapping strategies from family-based linkage approaches to population-based studies, primarily centered on unrelated case and control subjects. In identifying multiple common variants with modest effect (i.e., effect size generally less than 1.4) on its risk, these studies have improved our overall understanding of the allelic architecture that underlies DN and provided an alternative approach to identifying genetic variants associated with DN that are potentially distinct from those identified using linkage-based approaches. In comparison to

other diseases, relatively few GWASs in DN have been published. Those that have been published have been largely under-powered and, to date, no strong DN susceptibility loci have emerged from these studies. Nonetheless, given the polygenic nature of this complex disease, we expect that as more data are generated, associations will be identified at multiple loci. As has been demonstrated in other diseases, these loci, however, will likely explain only a modest proportion of the overall heritability of DN, leaving much of its genetic basis yet to be defined.⁴⁵

Next-generation Sequencing and the Search for DN Genes in T2D

Genes that contain variants with a major effect (i.e., those with effect sizes greater than 2.0) on the risk of DN in T2D have not been identified by GWAS-based approaches. This is due to the small effect sizes (less than 1.4) attributable to common variants and the existence of other genetic factors in DN's risk and disease etiology. An important source of genetic variation that has not thoroughly been assessed through GWASs is variants located in the genome's coding regions. Coding variants may have important protein altering consequences that significantly affect a protein's function. In studies of rare forms of kidney disease, including nephrotic syndrome and familial focal segmental glomerulosclerosis, several missense and nonsense mutations have been identified in genes that play a critical role in the structure and/or proper functioning of the glomerular filtration barrier, including nephrin [*NPHS1*], podocin [*NPHS2*], actinin alpha 4 [*ACTN4*], transient receptor potential cation channel subfamily C [*TRPC6*], and phospholipase C, epsilon 1 [*PLCE1*) in families with multiple affected members.^{46–50} Similarly, studies of maturity-onset diabetes of the young (MODY) present additional examples where the ascertainment of large affected pedigrees has facilitated the identification of rare, highly penetrant variants that co-segregate with the disease and cause this form of non-insulin-dependent diabetes.⁵¹

While such progress has not yet been seen in DN, the recent emergence of next-generation sequencing technology (that allows large genomic regions or entire genomes to be sequenced rapidly and accurately) and advances in target enrichment technologies (that allow specific sub-regions of the genome to be selected for resequencing) are beginning to facilitate these efforts.

Because low-frequency disease predisposing variants are more common among affected relatives compared to unrelated individuals, we believe that family-based studies offer the best opportunity to detect the rare functional variants that contribute to DN susceptibility. In support of this, we recently initiated a family-based targeted resequencing project to comprehensively survey rare variants that underlie the linkage signals at several previously identified chromosomal loci shown to contribute to variation in urinary albumin excretion and renal function in the Joslin Study on the Genetics of Type 2 Diabetes collection.⁵² One-hundred twenty-six DN cases from 42 families with an excess of renal disease (approximately 3.0 cases per family) were selected for resequencing of the coding region of 361 protein-coding genes located at four genomic regions with evidence for linkage of urinary albumin excretion levels (chromosomes 5q, 7q, 21p, and 22q) and two genomic regions linked to variation in renal function (chromosomes 2q and 7p). To date, we have completed sequencing and analysis of 63 DN cases from 21 of the selected families.

In Table 4, we provide a summary of variants identified in 74 genes across the 20 megabasepair (Mb) region on chromosome 7p where we previously reported significant evidence of linkage with eGFR.¹³ In this preliminary dataset, we identified a total of 385 non-reference variants, including 42 novel variants (i.e., those not annotated in the current release of NCBI's SNP database (dbSNP build 135; www.ncbi.nlm.nih.gov/projects/SNP/))

or present in data from the 1000 Genomes Project (www.1000genomes.org/) and 170 (44.2%) non-synonymous SNPs (i.e., missense and nonsense).

Although the analysis of this data is still ongoing, to illustrate the utility of combining evidence from family-based linkage analysis with targeted sequencing to uncover rare functional genetic variants that may contribute to DN, additional data is presented for the collagen type XXVIII alpha 1 (*COL28A1*) (Figure 1); a gene located at position 7.36 to 7.54 Mb on chromosome 7 and a member of the extracellular matrix molecule family of collagens that is known to be expressed in kidney.⁵³ Our resequencing efforts identified 10 variants in *COL28A1*'s coding sequence, including 8 non-synonymous SNPs and 2 synonymous SNPs. Two of these have not previously been reported in dbSNP or in data from the 1000 Genomes Project. All 8 non-synonymous SNPs are present in data from 4,300 European-Americans included in the NHLBI's recent Exome Sequencing Project's (ESP, evs.gs.washington.edu/EVS). Two non-synonymous SNPs shown in Figure 1 (chr7:7458491 and chr7:7483333) are extremely rare in the ESP dataset (MAFs = 0.02% and 0.2%, respectively). Of particular interest, chr7:7483333 was observed in two T2D sibling with ESRD from a single family. Analysis of chr7:7483333 in all available members of this family is currently underway.

As we continue our analysis of all 361 genes across the six linkage regions in the entire Joslin Study on the Genetics of Type 2 Diabetes collection, we anticipate that this approach will allow us to identify variants in genes that contribute to variation in urinary albumin excretion and renal function decline in T2D at regions linked with each of these traits.

This preliminary analysis highlights some important concepts. First, next-generation resequencing coupled with target enrichment is an efficient and cost-effective approach to comprehensively interrogate tens to hundreds of genes within an area of known linkage. As their associated costs continue to come down, these technologies will become increasingly vital in identifying rare functional variants associated with complex disease. Second, while a far reaching benefit from the ESP is the recent development of commercial genotyping arrays that contain >250,000 to 319,000 rare putative functional exonic variants that were generated as part of this project, resequencing in specific populations provides a more extensive catalog of the variation that may be relevant to a particular disease. For example, among the 170 non-synonymous SNPs we identified in the chromosome 7p linkage region, only ~ 61% are represented on the Infinium HumanExome Beadchip that was derived from the ESP. While commercial exome genotyping arrays offer previously unavailable coverage of rare variants at a very reasonable cost, resequencing, though more costly, is the most comprehensive approach to assess genomic variation both across a region of interest and in the population of interest. Finally, as illustrated by our analysis of *COL28A1*, family-based approaches are well-designed to identify functional variants that are extremely rare in the general population but that are common among related affected individuals. Renewed interest in such studies will help define the spectrum of genetic variation that accounts for the heritability of DN in T2D and its related traits.

Conclusion

For more than 20 years, evidence in favor a genetic basis for the susceptibility of DN in T2D has provided a foundation for studies aimed at identifying the causal genes responsible for its development. During this period, strategies used to map genes for DN have been driven by our understanding of variation across our genome and the technologies available to interrogate it; as both have evolved, so to have our approaches. The advent of next-generation sequencing technology and increased interest in the search for rare variants has begun to swing the pendulum of these efforts away from population-based studies and back

to studies of pedigrees. As the field moves forward, family-based approaches should greatly facilitate efforts to identify variants in genes that have a major affect on the risk of DN in T2D. To be successful, the ascertainment and comprehensive study of families with multiple affected members is critical.

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KEY POINTS

- Evidence in favor a genetic basis for the susceptibility of DN in T2D has provided a foundation for studies aimed at identifying the causal genes responsible for its development.
- During this period, strategies used to map genes for DN have been driven by our understanding of variation across our genome and the technologies available to interrogate it; as both have evolved, so to have our approaches.
- The advent of next-generation sequencing technology and increased interest in the search for rare variants has begun to swing the pendulum of these efforts towards studies of pedigrees.
- Family-based approaches should greatly facilitate efforts to identify variants in genes that have a major affect on the risk of DN in T2D. To be successful, the ascertainment and comprehensive study of families with multiple affected members is critical.

Figure 1. Coding variants in *COL28A1* in DN patients from the Joslin Study on the Genetics of Type 2 Diabetes collection

Ten variants identified in *COL28A1*'s coding sequence are shown, including 8 non-synonymous SNPs (red) and 2 synonymous SNPs (black). The rs identification numbers for variants reported in dbSNP build 135 are provided. Variants not in dbSNP build 135 are identified by their chromosome and position relative to NCBI Build 36.1.

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Table 1

Summary of Loci with Significant Evidence of Linkage with DN in T2D (MLS 3.3, $P < 4.9 \times 10^{-5}$).

Chr	Position (Mb)*	MLS/P-value	Phenotype	Population	Study Design	Reference
1	11.60–36.00	3.81	eGFR	European- and African-American	Families	Freedman et al. ²¹
1	233.96–242.13	3.78	eGFR	Mexican-American	Sibpair	Schelling et al. ²³
2	44.70–66.60	4.31	eGFR	European- and African-American	Families	Freedman et al. ²¹
2	195.00–213.00	4.1	eGFR, T2D relatives	Primarily European (94%)	Sibpair	Placha et al. ¹³
3	103.66–118.89	4.55	early-onset ESRD	African-American	Sibpair	Bowden et al. ^{17,†}
5	41.07–67.27	3.4	ACR	European-American	Families	Krolewski et al. ¹¹
7	6.00–26.00	4.0	eGFR, all relative-pairs	Primarily European-American (94%)	Sibpair	Placha et al. ¹³
7	151.20–154.20	4.23	eGFR	Mexican-American	Sibpair	Schelling et al. ²³
8	66.23–87.24	8.7×10^{-6}	eGFR	Mexican-American	Sibpair	Schelling et al. ²³
10	85.00–101.00	3.6	eGFR	European-American	Sibpair	Placha et al. ¹³
14	53.44–69.29	2.0×10^{-5}	Proteinuria/ESRD	American Indian	Primarily sibpair	Iyengar et al. ²⁰
16	74.60–86.50	3.56	Creatinine Clearance	West African	Sibpair	Chen et al. ¹⁹
18	56.97–71.24	3.72	ESRD, early-onset T2D	African-American	Sibpair	Bowden et al. ^{17,†}
18	68.95–73.23	6.1	Proteinuria	Turkish	Families	Vardarli et al. ¹⁶
18	71.24–75.95	6.4×10^{-6}	eGFR	Mexican-American	Sibpair	Schelling et al. ²³
22	27.19–35.08	3.7	ACR	Primarily European-American (94%)	Families	Krolewski et al. ¹¹

* Approximate positions for reported LOD-1 intervals are provided in mega-basepairs (Mb) relative to NCBI Build 36.1. For studies not reporting LOD-1 intervals, the approximated position of the peak/flanking markers is provided if available.

[†] Bowden et al.¹⁷ also identified 1 significant (LOD= 3.59, chromosome 7p) and 2 suggestive loci (LOD=2.94, chromosome 12 and LOD=2.85, chromosome 16) in OSA among ESRD patients with long duration of DM, however these findings could potentially be spurious.⁵⁴

MLS, maximum LOD score

T2D, type 2 diabetes

DN, diabetic nephropathy

ESRD, end-stage renal disease

ACR, albumin-to-creatinine ratio

eGFR, estimated glomerular filtration rate

Table 2

Summary of Loci with Suggestive Evidence of Linkage ($3.3 > \text{MLS} \geq 2.0$, $4.9 \times 10^{-5} < P < 7.4 \times 10^{-4}$).

Chr	Position (Mb)*	MLS/P-value	Phenotype	Population	Study Design	Reference
1	237.00	2.00	DN	American-Indian	Primarily Sibpair	Igo et al. ²²
2	50.69–68.09	3.02	eGFR	Mexican-American	Sibpair	Schelling et al. ²³
2	146.24	2.04	ACR	African-American	Primarily Sibpair	Igo et al. ²²
2	213.38–236.13	2.7	eGFR	Mexican-American	Families	Puppala et al. ¹⁸
3	71.61	2.76	ACR	African-American	Primarily Sibpair	Igo et al. ²²
3	118.89	2.52	ESRD, early-onset T2D	African-American	Sibpair	Bowden et al. ¹⁷
3	138.00–152.00	2.2	eGFR, non-T2D relatives	Primarily European-American (94%)	Sibpair	Placha et al. ¹³
3	188.62–198.53	2.21	Serum creatinine	West African	Sibpair	Chen et al. ¹⁹
6	10.63	2.84	DN	European-American	Primarily Sibpair	Igo et al. ²²
6	102.68–150.80	2.08	eGFR	West African	Sibpair	Chen et al. ¹⁹
7	8.00	2.00	DN	American-Indian	Primarily Sibpair	Igo et al. ²²
7	29.66–96.38	2.42	eGFR	European and African-American	Families	Freedman et al. ²¹
7	68.19–82.63	6.0×10^{-4}	ACR	European-American	Primarily Sibpair	Iyengar et al. ²⁰
7	82.63–95.90	6.0×10^{-5}	Proteinuria/ESRD	African-American	Primarily Sibpair	Iyengar et al. ²⁰
7	91.34	2.96	ACR	European-American	Primarily Sibpair	Igo et al. ²²
7	131.93–134.76	2.73	DN	Pima Indian	Sibpair	Imperatore et al. ¹⁵
7	151.20–155.68	3.1	ACR	Primarily European-American (94%)	Families	Krolewski et al. ¹¹
9	78.97–90.00	2.9	eGFR	Mexican-American	Families	Puppala et al. ¹⁸
10	4.87	2.10	DN	American-Indian	Primarily Sibpair	Igo et al. ²²
10	43.37–92.40	2.53	Serum creatinine	West African	Sibpair	Chen et al. ¹⁹
10	129.43	2.65	DN, late-onset T2D	African-American	Sibpair	Bowden et al. ¹⁷
11	11.00	2.28	DN	Mexican-American	Primarily Sibpair	Igo et al. ²²
11	17.04–24.08	2.3	eGFR	African-American	Sibpair	Schelling et al. ²³
11	36.00–53.00	2.1	eGFR, non-T2D relatives	Primarily European-American (94%)	Sibpair	Placha et al. ¹³
11	122.14–133.61	2.4	eGFR	Mexican-American	Families	Puppala et al. ¹⁸
12	16.91	2.86	ESRD, late onset T2D	African-American	Sibpair	Bowden et al. ¹⁷
12	113.58–127.91	2.69	eGFR	European-American	Families	Freedman et al. ²¹

Chr	Position (Mb)*	MLS/P-value	Phenotype	Population	Study Design	Reference
13	26.22–39.84	2.28	eGFR	European and African-American	Families	Freedman et al. ²¹
15	45.46	2.04	DN	American-Indian	Primarily Sibpair	Igo et al. ²²
15	63.13–68.97	2.98	eGFR	American Indian	Sibpair	Schelling et al. ²³
16	55.67	2.31	ACR	African-American	Primarily Sibpair	Igo et al. ²²
17	0.00–14.60	2.08	Creatinine clearance	West African	Sibpair	Chen et al. ¹⁹
18	6.00–10.00	2.2	eGFR, T2D relatives	Primarily European-American (94%)	Sibpair	Placha et al. ¹³
19	6.06	3.13	ESRD, late-onset T2D	African-American	Sibpair	Bowden et al. ¹⁷
20	17.32	2.5	ESRD, late-onset T2D	African-American	Sibpair	Bowden et al. ¹⁷
21	20.57	2.3	ACR	European-American	Families	Krolewski et al. ¹¹
21	46.86	2.59	ESRD, late-onset T2D	African-American	Sibpair	Bowden et al. ¹⁷
22	35.20	2.29	ACR	Mexican-American	Primarily Sibpair	Igo et al. ²²

* Approximate positions for reported LOD-1 intervals are provided in mega-basepairs (Mb) relative to NCBI Build 36.1. For studies not reporting LOD-1 intervals, the approximated position of the peak/flanking markers is provided if available.

MLS, maximum LOD score

T2D, type 2 diabetes

DN, diabetic nephropathy

ESRD, end-stage renal disease

ACR, albumin-to-creatinine ratio

eGFR, estimated glomerular filtration rate

Table 3

Summary of Loci Identified Through Genome-wide Association Analyses.

Chr	Position (Mb)*	SNP	Gene	Phenotype	Population	P-value	Reference
6	133.19	rs7769051 [†]	<i>RPS12</i>	ESRD	African-Americans (1,674 ESRD cases and 1,246 normalalbuminuric controls)	3.60×10 ⁻³	McDonough et al. ³⁷
6	148.75	rs6930576 [†]	<i>SASH1</i>	ESRD	African-Americans (1,674 ESRD cases and 1,246 normalalbuminuric controls)	5.30×10 ⁻³	McDonough et al. ³⁷
7	36.89	rs741301	<i>ELMO1</i>	Proteinuria/ESRD	Japanese (94 proteinuric/ESRD cases and 94 normalalbuminuric controls)	8.0×10 ⁻⁶	Shimazaki et al. ³⁹
8	129.14	rs2648875	<i>PVT1</i>	ESRD	Pima Indians (105 ESRD cases and 102 normalalbuminuric/microalbuminuric controls)	2.0×10 ⁻⁶	Hanson et al. ³⁸
9	93.02	rs773506 [†]	<i>AUH</i>	ESRD	African-Americans (1,674 ESRD cases and 1,246 normalalbuminuric controls)	2.57×10 ⁻⁴	McDonough et al. ³⁷
12	64.40	rs2358944 [†]	<i>MSRB3/HMGA2</i>	ESRD	African-Americans (1,674 ESRD cases and 1,246 normalalbuminuric controls)	4.70×10 ⁻³	McDonough et al. ³⁷
22	29.98–30.23	rs2106294, rs4820043, rs5749286 [†]	<i>LMK2/SF11</i>	ESRD	African-Americans (1,674 ESRD cases and 1,246 normalalbuminuric controls)	1.62×10 ⁻² –2.74×10 ⁻²	McDonough et al. ³⁷

* Approximate positions for leading SNPs in mega-basepairs (Mb) relative to NCBI Build 36.1.

[†] In combined analyses of 1,674 T2D ESRD cases and 1,719 non-T2D controls, these SNPs achieved *p*-values < 1.0×10⁻⁵.
ESRD, end-stage renal disease

Table 4

Summary of Variants Identified in the Chromosome 7p Linkage Region.

Total Number of Variants:	385
Number of Known Variants:	343
Number of Novel Variants:	42
Variant Class	<i>N</i>
Missense	162
Synonymous	80
Intronic	79
Downstream	36
Nonsense	8
Frameshift	7
Codon deletion	4
Stop lost	3
Codon insertion	2
Splice acceptor/donor	2
5' UTR	1
Start lost	1