

Diabetic Nephropathy: Is ESRD Its Only Heritable Phenotype?

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Genetic susceptibility contributes to the overall risk of nephropathy among individuals with diabetes. In this issue of *JASN*, Sandholm *et al.*¹ present the results of a sex-specific genome-wide association study aimed at identifying genetic factors for ESRD in type 1 diabetes. In identifying evidence of an association with ESRD exclusively in women, this study offers the strongest evidence to date of a sex-specific genetic factor for diabetic nephropathy. Importantly, this study also highlights an emerging shift in the strategy to search for diabetic nephropathy genes.

The conventional model of diabetic nephropathy, established more than 30 years ago, regards this complication as a progressive disease that advances sequentially through characteristic stages that are defined by increasing levels of urinary albumin excretion with the subsequent development of renal decline and ESRD.² Data from several longitudinal studies reported in the 2000s, including several from our group,^{3–5} began to show that the true evolution of diabetic nephropathy differs from this model. In fact, it has been shown that patients with albuminuria can frequently revert to normoalbuminuria. Only a minority of patients with albuminuria experiences renal decline that leads to ESRD. Furthermore, this decline varies widely among patients with type 1 diabetes.⁶ For some patients, renal function declines so slowly that ESRD might be reached only after 30 to 50 years of diabetes. For others, their decline in renal function is so rapid that they can progress from normal renal function to ESRD within as few as 2–5 years.⁶

These studies have begun to reshape our perception of the natural course of diabetic nephropathy and have implications for etiological studies as well as the development of intervention programs.⁷ They also have profound implications on genetic studies of diabetic nephropathy while raising several important questions that have yet to be answered. Where is the genetic contribution to diabetic nephropathy? Does it impact the development of urinary albumin excretion abnormalities (predisposition to micro- and macroalbuminuria)? Does it impact renal function decline (predisposition to progression from albuminuria to ESRD)? Does it impact the rate of renal

function decline (estimated GFR loss) that determines the time to onset of ESRD?

Most studies aimed at uncovering the genetic susceptibility of diabetic nephropathy, including our own study,⁸ continue to simultaneously consider a mosaic of case subjects (*e.g.*, individuals with varying levels of albuminuria or stages of CKD).^{9,10} Because each of these diabetic nephropathy “subphenotypes” most certainly differs with respect to the genetic etiology, it is not surprising that such studies have yielded relatively few reproducible genetic associations. To define the genetic factors that contribute to the susceptibility of diabetic nephropathy, it is critical that researchers recognize this inadequacy and design studies that use well characterized homogeneous patient cohorts. Because ESRD is a well defined, hard end point (as well as the cross-sectional nature of the majority of available patient collections), this subphenotype is presently the most pragmatic subphenotype to consider in genetic studies of diabetic nephropathy.

Expanding on a previous meta-analysis by the Genetics of Nephropathy - an International Effort (GENIE), in which no genome-wide significant associations were identified assuming the traditional model of diabetic nephropathy,¹¹ Sandholm *et al.*¹ choose to focus on ESRD as their phenotype of interest and further restrict their analysis by separately interrogating the association of common genetic variation and ESRD in men and women. Among 1193 type 1 diabetic women from the Finnish Diabetic Nephropathy (FinnDiane) study, a single genome-wide significant association ($P < 5 \times 10^{-8}$) at rs4972593 on chromosome 2q31.1 emerged from this analysis. This variant was not associated with the risk of ESRD in 1042 men from FinnDiane ($P = 0.77$), and modest evidence of replication ($P = 0.02$) was observed in three replication cohorts within the GENIE consortium. No other loci reached genome-wide significance in either men or women in FinnDiane. Although Sandholm *et al.*¹ provide limited *in silico* data suggesting that the minor allele of rs4972593 results in the loss of several transcription factor binding sites, the molecular mechanism underlying this association is largely speculative. This finding is potentially interesting if rs4972593 is proven to be the causative single nucleotide polymorphism (SNP) at this locus; however, it is noteworthy that the associated SNP is located within a large intergenic region and hundreds of kilobase pairs from the nearest gene.

This study represents the first large-scale, sex-specific examination of the association between genetic variation and the risk of ESRD in type 1 diabetes. We regard this study as a step forward in disentangling the genetics of diabetic nephropathy; nevertheless, these data should be interpreted in context. Below, we discuss several points we feel potential readers should consider as they evaluate the work presented by Sandholm *et al.*¹

First, are there sex differences in the incidence of ESRD in diabetic patients and, if so, does genetic variation account for these differences? In general, the incidence and rate of progression of non-diabetic kidney diseases are higher in men compared to women; in the setting of diabetes, however, data regarding the effect of sex on the risk of kidney disease has been

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inconclusive.¹² As suggested in a recent longitudinal study of participants of FinnDiane, such effects seem to be highly dependent on the age at onset of diabetes.¹³ In this study, no difference in the 30-year cumulative risk of ESRD between men and women existed when all patients were analyzed simultaneously.¹³ Interestingly, however, when the study stratified patients based on their age at onset of diabetes, sex-related differences in the risk of ESRD became apparent.¹³ Specifically, among patients diagnosed at 10 years of age or older, the risk of ESRD in men was two times the risk in women. In contrast, no sex-related difference was observed in patients diagnosed before age 10 years. These data suggest that the subgroup of women with an early age of diagnosis (<10 years) seems to lose the protective advantage against developing ESRD that women with a later age of diagnosis hold over men. In the study by Sandholm *et al.*,¹ the impact of age at diagnosis was not evaluated.

Second, although Sandholm *et al.*¹ restricted their analysis to cases with ESRD, as discussed earlier, not all patients who reach this end point do so by the same trajectory. Constitutional susceptibility to variation in this decline of renal function is likely to be caused by genetic predisposition. It is possible that multiple genes and multiple variants within these genes likely account for the large variation in the rate of renal function decline that is observed among individuals with type 1 diabetes. Whether genetic variants that influence this decline do so in a sex-specific manner, remains to be seen. Similarly, it is unclear whether the variant identified by Sandholm *et al.*¹ has any impact on the rate of renal function decline.

Third, additional evidence to replicate these findings is critical. A major limitation affecting this study and nearly all other genetic studies of diabetic nephropathy is the paucity of appropriate collections for study. Indeed, in the discovery cohort in this study, the power to detect genome-wide significant associations with the risk of ESRD in women was only 8%. In men, this study had 12% power. Because this study was not well powered to discover genetic variants that influence the risk of ESRD in men and women in a sex-specific manner, associations in addition to the association observed at rs4972593, if they exist, are likely to have gone undetected because of insufficient sample size in FinnDiane. Along this same line, the replication data presented by Sandholm *et al.*¹ were primarily driven by the US Genetics of Kidneys in Diabetes (GoKinD) collection. Both the Ireland-Warren 3-Genetics of Kidneys in Diabetes United Kingdom (UK-ROI) and Italian collections failed to achieve statistical significance, despite the UK-ROI collection being well powered. Reflective of this finding, when combined in a meta-analysis, the association at rs4972593 was attenuated. Once these findings are convincingly replicated, to fully understand the role of the reported association on the risk of ESRD in type 1 diabetes, the onus will then shift to carefully examining the biology underlying this association.

Fourth, could the variant identified by Sandholm *et al.*¹ purely be associated with increased survival among women with ESRD rather than risk of ESRD? Although not addressed

in this study, it remains possible that sex-specific competing risks could allow more women to survive ESRD than men. If true, the variant identified in this study may be a consequence of its association with sex-specific survival of ESRD rather than sex-specific risk for ESRD.

In conclusion, we feel that the study by Sandholm *et al.*¹ reflects an emerging shift in the strategy that investigators are using to search for diabetic nephropathy susceptibility genes. Investigators are clearly beginning to better align study designs with our current understanding of the natural history of diabetic nephropathy. As more studies in homogeneous, well phenotyped collections are reported, it is our belief that the genetic etiology underlying the risk of diabetic nephropathy and the subphenotypes that contribute to its development will be revealed.

DISCLOSURES

None.

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See related article, “Chromosome 2q31.1 Associates with ESRD in Women with Type 1 Diabetes,” on pages 1537–1543.

Induced Pluripotent Stem Cells from Polycystic Kidney Disease Patients: A Novel Tool to Model the Pathogenesis of Cystic Kidney Disease

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Polycystic kidney disease (PKD) is the most common inherited kidney disorder in humans, affecting 1 in 400–

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1000 live births. PKD can be inherited as an autosomal dominant (ADPKD) or autosomal recessive trait (ARPKD). ARPKD is characterized by a rapid progressive course of disease with childhood kidney failure. The defective three-dimensional tissue organization in ADPKD also begins *in utero* but progresses slowly, and signs of the disease may not be detected until late adulthood. The severity of the renal disease in ADPKD is highly variable, ranging from rare *in utero* cases with massively enlarged cystic kidneys to cases with adequate kidney function into old age. Although the two disorders present with different dynamics, their pathology eventually converges on ESRD.¹

ADPKD is caused by mutations in *Polycystic Kidney Disease* (*PKD1*) or *PKD2*, whereas ARPKD arises from mutations in *Polycystic Kidney and Hepatic Disease 1* (*PKHD1*; #173,900, #613,095, and #263,200 in Online Mendelian Inheritance of Man, <http://www.ncbi.nlm.nih.gov/omim/>). *PKD1* encodes for polycystin-1 (PC1), *PKD2* encodes for polycystin-2 (PC2; also known as transient receptor potential PC2, TRPP2), and *PKHD1* encodes for fibrocystin/polyductin (FPC). A high level of allelic heterogeneity is found for all PKD genes, with hundreds of unique mutations reported for ADPKD and ARPKD (*PKD1*, 1923 reported mutations; *PKD2*, 241 reported mutations; *PKHD1*, 713 reported mutations; The ADPKD Mutation Database [<http://pkdb.mayo.edu>] and The ARPKD/PKHD1 Mutation Database [<http://www.humgen.rwth-aachen.de/index.php>]). The vast majority of mutations is unique to single families, with alleles ranging from predicted loss of function to hypomorphic variants. To date, there is rather limited information on genotype–phenotype correlation. It has been particularly difficult to discriminate between pathogenic nucleotide substitutions and harmless sequence variants based on *in silico* predictions.

Multiple approaches have been used to define the functional connection between PKD disease genes and PKD disease phenotypes. The most significant progress, including the identification of causative genes and the discovery of the importance of cilia, has been achieved through genetic approaches.² The major remaining challenge centers on understanding the *in vivo* functions of the polycystins at the cellular and molecular levels. This undertaking is complicated by two factors. (1) The immediate signaling effectors downstream of polycystins have not yet been identified. (2) PKD is a disease of three-dimensional organ structure, and it is not clear whether *in vitro* cell-based models can recapitulate polycystin function *in vivo*. *In vitro* assays to quantify the function of PKD gene products in a robust fashion are urgently needed to investigate the molecular pathogenesis of PKD and determine the functional impact of distinct patient mutations.

In the current issue of *JASN*, Freedman *et al.*³ report the generation and characterization of induced pluripotent stem cells (iPSCs) from patients with ADPKD and ARPKD. To model PKD in human cells, Freedman *et al.*³ established iPSCs