# Genetics of Diabetic Nephropathy: Lessons from Mice

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Diabetic nephropathy (DN) is the single major cause of end stage renal disease in the US. DN only occurs after years of hyperglycemia and characterized by progressively increasing albuminuria, declining glomerular filtration rate, and increased cardiovascular risk. Only a minority of diabetics (25-40%) develop nephropathy, and there is evidence that heritable genetic factors predispose these "at-risk" individuals to DN. Comparing variability among inbred mouse strains with respect to a specific phenotype can model inter-human variability, and each strain represents a genetically homogenous system with a defined risk of nephropathy. We compared diabetic nephropathy in six inbred mouse strains including C57BL/6J, DBA/2J, FVB/NJ, MRL/MpJ, A/J, and KK/HlJ mice. Despite comparable levels of hyperglycemia in streptozotocin treated mice, urinary albumin excretion and renal histopathological changes were dramatically different between strains. DBA/2J and KK/HlJ mice developed significantly more albuminuria than C57BL/6J, MRL/MpJ or A/J mice. A progressive and significant decline in GFR was evident after the 52-weeks of hyperglycemia in DBA/2J mice

To define the genetic factors that protect C57BL/6 mice from DN we use N-ethyl-N-nitrosourea (ENU), to mutagenized male B6 mice and screened progeny for mutants developing excess albuminuria on a sensitizing type 1 diabetic background contributed by the dominant Akita mutation in insulin2 gene ($Ins2^{Akita}$). Two out of 375 diabetic 1$^{st}$ generation G1 founders exhibited albumin excretion rates (AER) persistently 10 fold greater than AERs in non-mutagenized diabetic $Ins2^{Akita}$ controls. This albuminuric trait was heritable and transmitted to ~50% of $Ins2^{Akita}$ G2 and G3 progeny, consistent with a simple, dominantly inherited trait. After a year of age, albuminuric $Ins2^{Akita}$ G2 and G3 progeny exhibited reduced inulin clearance, with elevated BUN and plasma creatinine. Glomerular histology revealed mesangial expansion, and glomerular basement membrane thickening determined by electron microscopy was enhanced in diabetic mutant kidneys.

Finally we characterized the role of candidate genes in the development of murine DN. Functionally significant polymorphisms in endothelial nitric oxide synthase (eNOS) and altered vascular eNOS activity have been associated with accelerated human diabetic nephropathy (DN), but the causative data implicating eNOS deficiency in the development of DN has not previously been established. We backcrossed $eNOS^{-/-}$ mice with C57BLKS/J *db/db* mice and investigated the renal phenotype. Although the severity of hyperglycemia was similar to C57BLKS/J *db/db* mice, by 26 weeks, $eNOS^{-/-}$ C57BLKS/J *db/db* mice developed hypertension, dramatic albuminuria, arteriolar hyalinosis, increased GBM thickness, mesangial expansion, mesangiolysis and focal segmental and early nodular glomerulosclerosis. Even more remarkably, $eNOS^{-/-}$ C57BLKS *db/db* exhibited increased serum creatinine and decreased glomerular filtration rates to less than 50% of $eNOS^{+/+}$ C57BLKS *db/db*.

In summary, utilization of unique genetic reagents available in mice provides evidence support a genetic predisposition to diabetic nephropathy. Roles for both recessive modifiers (e.g. eNOS) and dominant modifiers (ENU induced mutations) are supported. Identification of genetic risk factors predisposing to diabetic nephropathy in mice should provide novel targets for the treatment of DN.