# Diabetic Complications Consortium

**Application Title:** COL4A3 Variants and Susceptibility to Diabetic Nephropathy

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## 1. Project Accomplishments:

The overall goal of this project was to investigate whether a common variant (D326Y) in the collagen α3(IV) (COL4A3) protein discovered in a GWAS analysis as protective against diabetic nephropathy in patients with type I diabetes is also protective in mice. If so, this would greatly facilitate future mechanistic studies. The approach was to use CRISPR/Cas9 to alter the relevant nucleotides in the mouse *Col4a3* gene so that the protein would be either be D326 or Y326, mimicking the human variant proteins. The wild-type amino acid at that position in mice is A, so the human common D and less common Y each needed to be engineered.

We successfully generated the D and Y variants on the pure FVB/NJ background and on the mixed B6CBA background. We also identified deletions in *Col4a3* near the gRNA recognition site that should cause Alport syndrome due to loss of COL4A3.

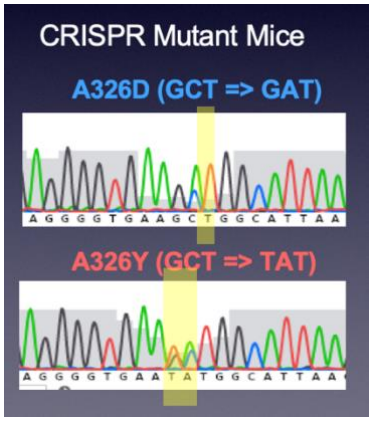
COVID-19 and the resulting mandated laboratory ramp down at about the time we expected to begin crossing the edited FVB/NJ mice onto the OVE26-FVB/NJ diabetic background led to a slowdown in progress towards generating these mice. Once breeding could be increased in early May, we learned about the significant difficulties in breeding the OVE26-FVB/NJ mice. Nonetheless, using in vitro fertilization, we have recently been able to generate OVE26-FVB/NJ mice carrying either Y326 or the wild-type A326. We are breeding to get more of these mice while the ones on the shelf continue to age so that we can eventually look for differences in level of albuminuria and GBM thickness.

## 2. Specific Aims:

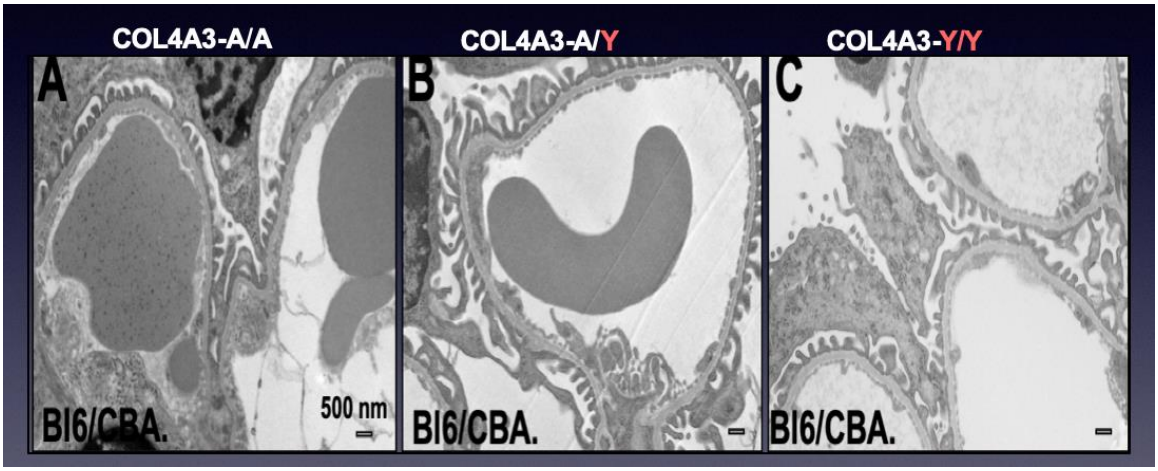
**Specific Aim 1:** Characterize the impact of the COL4A3-Y326 variant on GBM thickness in healthy mice.

**Results:** We successfully generated *Col4a3* mutant mice expressing either the D326 (common human) allele or the Y326 (less common human, protective) allele. The first figure below contains the sequence chromatograms that show that these changes were introduced. Note that A326 is the wild-type mouse allele that we believe should not be protective for diabetic nephropathy; instead, we hypothesize that it is the large tyrosine residue, not often found in COL4 collagenous domains, that should be protective when

present at amino acid position 326. We generated the A326D and A326Y mutants on both the pure FVB/NJ and mixed B6CBA strain backgrounds.

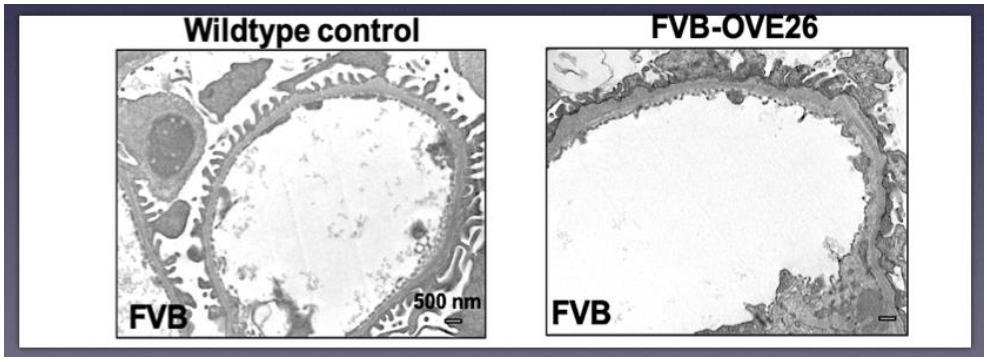



So far we have examined the ultrastructure of the glomerular filtration barrier of normoglycemic COL4A3-A/A, -A/Y, and -Y/Y mice at 24 weeks of age on the mixed B6CBA background. As shown in the figure below, there are no clear differences in the GBM thicknesses among these healthy control mice. We are in the process of generating D/D and D/Y mice for comparison, and will also examine these genotypes on the pure FVB/NJ background.




**Specific Aim 2:** Characterize the impact of the COL4A3-Y326 variant on GBM thickness in diabetic mice.

Although we initially proposed to use the DBA/2J strain background due to its susceptibility to STZ-induced diabetes and diabetic nephropathy, we decided to use the well characterized OVE26-FVB/NJ genetic model instead to avoid the side effects and variability of STZ. In the figure below, we analyzed the ultrastructure of healthy FVB and OVE26-FVB GBM and found that there is significant thickening of the OVE26 mouse GBM, as well as foot process effacement.



**Results:** As discussed under Project Accomplishments, due to the COVID-19-related slowdown in breeding and the inherent breeding problems with OVE26-FVB/NJ diabetic mice, we only recently generated a cohort of diabetic mice carrying the Y326 variant, and the variant is in the heterozygous state, with the other allele being the WT A326. We plan to analyze the level of albuminuria and the thickness of the GBMs when the mice are approximately 3 months old. We expect to find thinner GBMs in the diabetic A/Y mice vs. their A/A littermates.

## 3. Publications:

None