**Comments:**

First paragraph reads too much like a list. The words are stiff and lacks flow

**Title: TBD**

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Alport Syndrome (AS) is a hereditary disease affecting 1 in 5,000 births in the United States1. Children and young adults with AS suffer from hearing loss, vision abnormalities, and most notably kidney disease2. AS patients with kidney disease are diagnosed with glomerulonephritis, and present with symptoms of hematuria and proteinuria. Inevitably, their kidney functions progressively deteriorate and lead to End-Stage Renal Failure (ESRF) 2. It is well studied that ESRF in AS is caused by genetic mutations in the α3, α4, and α5 chains of type IV collagen, which are encoded by *COL4A3, COL4A4, and COL4A5*. In the kidney, the three type IV collagen proteins form heterotrimers and are exclusively found in the glomerular basement membrane (GBM)3. The lack of type IV collagen proteins causes the GBM to weaken and distend, and podocytes foot process effacement is also observed (REFERENCE). COL4A5 is the only type IV collagen protein encoded on the X chromosome, and is responsible for 80% of AS diagnosis. As the X-linkage suggests, males that are hemizygous to the *COL4A5* mutation are disproportionally affected compared to females. Males have an earlier onset and increased severity of the disease, with 50% of patients requiring dialysis or kidney transplants due to ESRF by the age of 25 and 100% by the age of 604. On the other hand females that are heterozygous for this X-linked AS have relatively later onset with only 12% developing ESRF by the age of 40 and 40% at the age of 80 years5. However, patients do not all present the disease in a similar manner, and studies have observed their age of onset and severity to be highly variable4.

In the 2015 International Workshop on AS, clinicians and researchers highlighted the need for an effective cure for AS6. Currently the only treatment option for AS patients is Angiotensin-converting-enzyme inhibitors (ACE-inhibitors), which is primarily used as treatment for hypertension4. Treatment with ACE-inhibitors are able to alleviate the mechanical pressures applied to the fragile GMB of AS patients and delay onset of ESRF, however treatment efficacy is highly dependent on timing6,7. In addition to the lack of treatment options, we still do not have a target for therapeutic interventions. Although *Col4a5* is known to cause AS, it is a poor therapeutic target as the disease progression vary dramatically. The varying age of onset and severity of AS suggests possibilities of other underlying mechanisms that are able to modify disease progression caused by the *Col4a5* mutation. Identifying these mechanisms and the genes involved may lead to the development of precise and novel therapeutic targets.

To our knowledge there has only been one study conducted to identify modifier genes that lead to the variability in age of onset and severity of AS8. They observed variation between *Col4a3* knock out mice in 129X1/SvJ and C57BL/6J backgrounds, and identified 2 quantitative trail loci (QTL) on chromosome 9 and 16, however the intervals were not narrow enough to confidently identify probable candidate genes8.

In this study we aimed to identify modifier genes of X-linked AS by introducing the *Col4a5* mutation into a diverse genetic background using the Diversity Outbred (DO) mice 9-11. DO mice are derived from eight inbred founder strains (A/J, C57BL/6J, 129S1/SvImJ, NOD/LtJ, NZO/HlLtJ, CAST/EiJ, PWK/PhJ, and WSB/EiJ), which captured nearly 90% of the known genetic variation present in laboratory mice12. This heterogeneity observed in the DO population is currently the best model that reflects the genetic diversity in the human population. (Ending pending results)

1. Thomassen M, Flinter F. Clinical utility gene card for: Alport syndrome. *Eur J Hum Genet*. 2012;20(6):–. doi:10.1038/ejhg.2011.237.

2. Savige J, Gregory M, Gross O, Kashtan C, Ding J, Flinter F. Expert guidelines for the management of Alport syndrome and thin basement membrane nephropathy. *J Am Soc Nephrol*. 2013;24(3):364-375. doi:10.1681/ASN.2012020148.

3. Hudson BG, Tryggvason K, Sundaramoorthy M, Neilson EG. Alport“s syndrome, Goodpasture”s syndrome, and type IV collagen. *N Engl J Med*. 2003;348(25):2543-2556. doi:10.1056/NEJMra022296.

4. Kashtan CE, Rheault M. Clinical practice recommendations for the treatment of Alport syndrome: a statement of the Alport Syndrome Research Collaborative. *Pediatr Nephrol*. 2013;28(1):5-11. doi:10.1007/s00467-012-2138-4.

5. Jais JP, Knebelmann B, Giatras I, et al. X-linked Alport syndrome: natural history and genotype-phenotype correlations in girls and women belonging to 195 families: a “European Community Alport Syndrome Concerted Action” study. *Journal of the American Society of Nephrology*. 2003;14(10):2603-2610. doi:10.1097/01.ASN.0000090034.71205.74.

6. Flinter F, Savige J, Savva I, et al. Advances and unmet needs in genetic, basic and clinical science in Alport syndrome: report from the 2015 International Workshop on Alport Syndrome. *Nephrol Dial Transplant*. May 2016:gfw095. doi:10.1093/ndt/gfw095.

7. Gross O, Licht C, Anders HJ, et al. Early angiotensin-converting enzyme inhibition in Alport syndrome delays renal failure and improves life expectancy. *Kidney International*. 2012;81(5):494-501. doi:10.1038/ki.2011.407.

8. Andrews KL, Mudd JL, Li C, Miner JH. Quantitative trait loci influence renal disease progression in a mouse model of Alport syndrome. *Am J Pathol*. 2002;160(2):721-730. doi:10.1016/S0002-9440(10)64892-4.

9. Churchill GA, Gatti DM, Munger SC, Svenson KL. The Diversity Outbred mouse population. *Mamm Genome*. 2012;23(9-10):713-718. doi:10.1007/s00335-012-9414-2.

10. Bogue MA, Churchill GA, Chesler EJ. Collaborative Cross and Diversity Outbred data resources in the Mouse Phenome Database. *Mamm Genome*. 2015;26(9-10):511-520. doi:10.1007/s00335-015-9595-6.

11. Gatti DM, Svenson KL, Shabalin A, et al. Quantitative trait locus mapping methods for diversity outbred mice. *G3 (Bethesda)*. 2014;4(9):1623-1633. doi:10.1534/g3.114.013748.

12. Threadgill DW, Churchill GA. Ten years of the collaborative cross. *G3 (Bethesda)*. 2012;2(2):153-156. doi:10.1534/g3.111.001891.