**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 disease2,3. 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) 3-5. 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) 6,7. A dysfunction in any one of the type IV collagen proteins causes the GBM to weaken and distend, and podocytes foot process effacement to occur5. 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 608. On the other hand females that are heterozygous for this X-linked AS have relatively later onset with 12% developing ESRF by the age of 40 and 40% at the age of 80 years9. Curiously, patients with similar genetic mutations do not all present the disease in a similar manner, and studies have observed their age of onset and severity to be highly variable8.

In the 2015 International Workshop on AS, clinicians and researchers highlighted the need for an effective cure for AS10. Currently the only available treatment options for AS patients are Angiotensin-converting-enzyme inhibitors (ACE-inhibitors) or angitotensin receptor blockers (ARBs), which are primarily used as treatment for hypertension2,8. 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 timing10,11. In addition to the lack of treatment options, we still do not have a specific target for therapeutic interventions. Although *Col4a5* is known to cause AS, it is a poor therapeutic target as patients with this mutation vary dramatically in disease progression. It is widely accepted that the varying age of onset and severity of AS is in part due to underlying mechanisms that are able to modify disease progression10,12,13. However, small samples sizes and other confounding factors preclude the ability to study such modifier in humans.

To our knowledge there has only been one study conducted to identify modifier genes in AS models12. They observed variations 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 candidate genes12. Since 2002, when the prior mentioned study was conduction, there have been major technological advancements and recourses to allow for high-resolution mapping. The Diversity Outbred (DO) mouse population, first published in 2012, is a genetically heterogeneous model derived from multi-parent crosses consisting of 5 classical inbred models (A/J, C57BL/6J, 129S1/SvImJ, NOD/ShiLtJ, and NZO/HlLtJ) and 3 wild-derived models (CAST/EiJ, PWK/PhJ, and WSB/EiJ)14. Each individual mouse in a DO population is a genetically unique combination of the 8 founder strains, and best reflects the diversity seen in human populations14,15. Furthermore, the development of the third generation of the Mouse Universal Genome Array series, GigaMUGA, allow for high-resolution mapping at 143,259 SNPs, and the ability to detect parental origin haplotypes in a DO population16. Utilizing these aforementioned recourses with enough sample size would be ideal for mapping modifier genes in AS.

In this study we aimed to effectively identify modifier genes in X-linked AS by introducing the *Col4a5* mutation into a diverse geneti­­­c background using the DO mouse model. The founder strains that make up DO mice contribute 90% of known genetic variations found in laboratory mice, and the captured genetic variations are randomly distributed across the genome17,18. Each F1 mouse with an X-linked AS mutation influenced by a unique combination of heterogeneous genetic background will present a range of renal phenotypes representative of the human AS population. With a sufficient samples size through this method, we will have the ability for high-resolution mapping, and identify modifier genes in X-linked AS. The identification of modifier genes will allow for the design of targeted therapeutics, an intervention urgently needed in for AS patients around the world.

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