**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 proteinuria4,5. Inevitably, their kidney function progressively deteriorates and leads to End-Stage Renal Failure (ESRF) 3,6,7. 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*2,4,8-10. In the kidney, the three type IV collagen proteins form heterotrimers and are exclusively found in the glomerular basement membrane (GBM) 8,10,11. A dysfunction in any one of the type IV collagen proteins causes the GBM to weaken and distend, and podocytes foot process effacement to occur7. COL4A5 is the only type IV collagen protein encoded on the X chromosome, and is responsible for 80% of AS diagnoses5. 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 6012. 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 years5,13.

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 variable12. Clinical outcomes of patients with similar familial mutations that present with hematuria are variable, ranging from normal renal function throughout life to chronic kidney disease to ESRF10,14. It is widely accepted that the variable clinical outcomes such as the age of onset and severity of AS is in part due to underlying genetic mechanisms that are able to modify disease progression5,15-17. Such genetic mechanisms are hereinafter termed modifier genes. Modifier genes act as independent genes that have the ability to affect the expressivity of phenotypes to either extremes18, and in the case of AS, the age of onset and severity. Studies to map modifier genes in humans AS patient cohorts have been attempted, however due to variable environmental factors, insufficient sample size, and other confounding factors make it difficult to draw confident conclusions19-21. For these reasons mammalian AS models such as mice would be more appropriate as their external environment, genetic background, and sample size can be controlled to meet the needs of an experimental design.

To our knowledge there has only been one study conducted to identify modifier genes in AS models16. Andrews K. L. et al. observed variations in phenotype between *Col4a3* knock out mice in a 129X1/SvJ, C57BL/6J, F1 cross between 129X1/SvJ and C57BL/6J, and a F1 to C57BL/6J back cross mouse models, which highlighted the presence of modifier genes (explain more).

and identified 2 quantitative trail loci (QTL) on chromosome 9 and 16, however the intervals were not narrow enough to confidently identify candidate genes16. 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)22. 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 populations22,23. 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 population24. 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 genome25,26. 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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