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Living With It: A Patient's and a Biochemist's Perspective on Kidney Disease

A Historical Review of Alport Syndrome

WWU Honors Program Senior Capstone Project

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Introduction

Kidney disease in humans is such a prevalent issue, especially with regards to aging populations, that there is a distinct qualifier for Medicare (in the United States) made just for patients with end-stage renal disease (ESRD). Chronic kidney disease is so prevalent, in fact, that even though medical technology has advanced greatly in the last few decades, clinically-defined kidney disease has only fallen from over 10.0% prevalence in all U.S. adults aged 20-65 down to 6.9% in adults as of 2016^{1,2}. Among all of these patients, some develop a loss in renal function through other medical conditions, such as diabetes, obesity, or hypertension, which has gained increased awareness in recent years. Others, however, come across their loss in function through genetics and mutations rather than from other health issues normally associated with more aged individuals. One such genetic function is called Alport Syndrome, which affects about 0.2% of adults, as well as about 3% of children in the U.S., around 1 in 5,000 births^{3,4}. Studies of Alport Syndrome (AS) at the genetic level have revealed much about the nature of this syndrome and about the mechanism of kidney disease as an umbrella term, starting well before the turn of the 21st century and continuing today. Herein is a report detailing a review of the history of such studies, major turning points in the progressive battle against AS, how clinical medicine has advanced as a result, and what the cutting edge of research means for the future of not only AS, but many other forms of kidney disease and other genetic diseases.

Initial Research - Finding Alport Syndrome

Chronic kidney disease, as a general blanket category of diseases, has been studied throughout modern history, with published medical studies identifying kidney syndromes or diseases dating back to the 19th century, with observations dating well before^{4,5}. While rudimentary for the most part, and lacking modern technologies or even simple light microscopy, such studies were able to direct observations toward a trend of symptoms linked to patients with kidney disease, such as proteinuria, hematuria and atrophied kidneys via postmortem autopsies³⁻¹². Strikingly, even though no thorough imaging or screening tests existed at the time, these consistent observations by Dr. Richard Bright, Domenico Cotugno, and others dating from the late 16th century onwards prompted additional study throughout the 19th and 20th centuries^{13,14}. At this point in history, forms of chronic kidney disease were identified due to the distinct excretion of protein and the formation of urinary sediment, such as uric acid crystallization^{13,14}. However, genetic diseases such as Alport Syndrome had not been documented yet, and would not be until more effective detection methods became available and genetic disease as a whole became a more relevant field of research, despite the results that Gregor Mendel published in 1866 that would set the standards for modern genetics, a now-famous experiment taught in every biology class¹⁵.

Today, most biology textbooks note that the studies published by Mendel went largely unnoticed until the turn of the 20th century, when methodology surrounding modern genetics was said to have been “rediscovered”. Namely, in conjunction with Friedrich Miescher’s discovery of DNA in 1869, independent study from three botanists by the names of Hugo DeVries, Carl Correns and Erich von Tschermak, were able to achieve results confirming the rules for genetic inheritance as observed by Mendel at the turn of the 20th century, and their papers publishing these findings helped greatly to solidify the base of what we know as modern genetics¹⁶⁻¹⁹. In the decade following the publication of these results, there was something of a revelation in this new field; the term “gene” was coined by Wilhelm Johannsen in 1909, and just

seven years earlier was the advent of observed genetic inheritance in the form of hereditary disease, along with publication of chromosome theory by Archibald Garrod and Walter Sutton respectively. Of course, we have now learned how lucky Mendel and his contemporaries were when it came to their observations of genetics in action; independent loci on autosomal chromosomes with only two distinct alleles in diploid organisms is a specific combination that can be observed as its own system because no cross-analysis must be performed with other variables, such as a third allele, locus, or a regulatory gene in another location entirely, let alone traits with incomplete or codominant phenotypic behavior or other inheritance patterns. While at this point researchers were aware that DNA existed and formed tightly packed chromosomes (though Watson, Crick, and Franklin had not started their work on elucidation of nucleic acid structure yet), nobody had attributed hereditary genes, or even traits generally, to a specific chromosome until Thomas Hunt Morgan published his study of fruit flies (*Drosophila melanogaster*) and directly linked expression of eye color to fruit flies' X chromosomes. The results of this one study were certainly unprecedented, and one consequence was the advent of techniques such as pedigree charts within families for scientists and counselors to deduce inheritance patterns over multiple generations with minimal information as opposed to genome sequencing, which would come about as a method far later into the 20th century. The aforementioned studies were all highly influential in allowing the discovery of Alport Syndrome, detailed herein.

Alport Syndrome, as described previously, is a genetic disorder that affects around 1 in every 5,000 individuals in the United States, and it is characterized by symptoms related to chronic kidney disease (CKD), such as proteinuria, hematuria, and in many cases symptoms of moderate hearing loss and moderate vision loss that manifest in development of ESRD, which can further be characterized by fatigue and high blood pressure³⁻¹². Additionally, modern metrics identify glomerulonephritis as another characterizing feature of Alport Syndrome¹². The

disease's first documented discovery was in 1927 by a researcher named Cecil A. Alport, for whom the disease is named, in one particular family that tended to display some, if not all of these aforementioned symptoms through multiple generations²⁰. This particular study was a combination of information provided by the family themselves, along with corroborating observations and relatively qualitative (rather than quantitative) data simply due to the time when this disease was discovered; after genetics had a foundation, but before sequence specificity or even nucleic acid structure had been elucidated. Fortunately for future researchers, this immediate family was observable over multiple generations rather than just one, which can sometimes hinder other case studies, and this is particularly important for this genetics study because it exhibits a *pattern* of inheritance that other types of studies, such as today's *in vitro* sequence modification can never account for. Results of this paper were crucial both for determining a mode of inheritance for this form of AS, as well as gathering a sense of scale in both symptom severity and in timescale for the way this then-novel condition progressed. For the case regarding this study, results pointed to an X-linked inheritance pattern virtually identical to the one observed in Morgan's *Drosophila* study, a notable attribute due to its continuation of sex-linkage theories stemming from Morgan's study. This is also important because this would turn out to be the most prevalent form of AS, present in around 85% of patients^{4,7,8,9,12}. However, since survival rates in patients were so low and onset of renal failure occurred so early for hemizygous males and phenotype expressing females with two mutant X chromosomes, AS was difficult to study. For these same reasons, it was difficult to develop treatment plans for such individuals simply because these efforts were more pioneering than doctoring, and as the history of treatments for chronic kidney conditions will show in the following section, advancements in such treatment technologies producing high success and survival rates are quite novel, indeed.

Seminal Research in Kidney Disease

In order to understand today's treatment methods, advancements, and explain why kidney disease is still so prevalent, it is beneficial to observe how kidney disease was diagnosed and treated in history. Given the aforementioned knowledge that kidney disease was relatively unknown in the realm of medicine apart from passing observations until the 18th and 19th centuries, more was discovered about the condition itself, including signs and symptoms, far before treatment even became an option, a theme fairly universal regarding all diseases. Even less would have been known to such contemporaries of the nature of heritable diseases. As previously described, genetics and heritability only made their way into mainstream science at the turn of the 20th century, and so in a sense the history of treatments for kidney disease is still quite young, and also quite novel. Still, though, methods for screening and diagnosing disease before attempting treatment are also important. For example, although observation of the urine by the naked eye has always been possible throughout civilization, the advent of microscopy allowed for a closer look at urine, and this is the first notable advancement in treatment technology for nephrologists; with the ability to see smaller details, observers as early as the late 16th century such as Nicolas-Claude Fabricius de Peiresc were able to describe crystals in urine that are now known to likely represent uric acid, though with poor resolution and poor focal lengths²¹. In a similar vein, the first descriptions of renal structure in general were elucidated by then-novel microscopy observations by Marcello Malpighi in 1666²¹. Briefly summarized, microscopy is regarded here as the first step towards a myriad of other methods, evolving as a method to accommodate the demands of other methodologies, from simple magnification lenses as employed in the 17th century to today's most advanced machines.

Before the 20th century, the greatest breakthroughs in medicine regarding chronic kidney disease, after the advent of the microscope, came from the works of scholars focused on the microbiological states and signs of kidney disease. Among this cohort are the minds of the aforementioned Richard Bright, as well as other pioneering scholars of clinical nephrology such

as William Bowman, John Bostock, Pierre Francois Rayer, and Friedrich Theodor von Frerichs, whom are related through their universal usage of urine and blood analysis through either macroscopy or microscopy, abetted by similar observations in biopsies and autopsies of patients^{21,22,23}. One of Bowman's crowning works, published in 1842, detailed his findings regarding the structure and function of a large portion of the glomerular capillary network in the membrane of the kidney. Namely, the structure (altogether called a nephron) understood then (and now) consists of a capillary network connected to distinct structures called renal tubules, which parse blood through a capsule subunit (later named after Bowman) in order to remove large macromolecules such as proteins²². Later, the last subunit of this network would be described by anatomist Friedrich Gustav Jacob Henle in 1866 as a subsequent set of tubule "loops" which serve to reintroduce filtered blood to the bloodstream and maintain homeostatic conditions in the glomerular matrix as well as the filtered blood^{22,24}. During the conduction of Bowman's and Henle's experiments, these other pioneers in clinical nephrology were altogether concerned with the pathology of the kidney; knowing that there was a diseased state and that it was observable, Bright's initial descriptions of macroscopic symptomatology related to kidney diseases were one of the most crucial first steps taken into the clinical field, all without usage of microscopy. Bright's work also delineated kidney diseases into two classes based on how "visible" the effects and symptoms were, though his admission that these classifications were rather arbitrary lend themselves to the notion that until microscopy was prevalent, even the most studied minds were held back by a lack of technology^{21,22}.

In addition to the advent of microscopy, a growing interest in cellular and organismal biology led to some other advancements in kidney research preceding the 20th century, one being the new prevalence of cell staining and the observation of renal tissue in addition to the urine. While Rayer's papers noted the first presence of microscopic hematuria, published from 1839-1841, and helped pave the way for the discoveries noted by Bowman and Henle, these

were only made possible by a technique developed in 1837 by physiology professor Gabriel Valentin. His technique would involve using carmine dyes to prepare thin layers of renal tissue on glass slides, colored to highlight structural patterns and the form of the tissue, and subsequently observed by microscopy these forms in a technique we know today as cell staining^{21,22}. In this initial version of the technique, the tissue could be trans-illuminated and the overall structure could be observed, including various artifacts of kidney substructures and abnormalities within these, such as accumulations of fats, proteins, or blood cells, now known as casts, in the aforementioned renal tubules²². In time, of course, this technique would be improved upon, and in 1869 Edwin Klebs' introduction of paraffin embedding would render methods using carmine and other cell dyes obsolete due to their inefficiency²². Additionally, like any novel medical technology, preliminary "trials" such as what are shown in the works of Valentin, Bowman, and Henle were precursors to the acceptance and introduction of cell staining to clinical and/or medical mainstream technologies; virtually all clinical studies to the point of 1850 and in that decade were performed with less-informative unstained tissues²².

One last important technology to take note of before the turn of the 20th century was the advent of centrifugation and its subsequent uptake by clinical researchers in a multitude of fields, including those concerned with renal disorders. Based on the idea of centrifugal forces, the methodology of separating substances using these forces did not first evolve in a clinical setting, but rather an agricultural one. A German brewer by the name of Antonin Prandtl had a predicament: when he was trying to separate cream from milk, he found the process of settling inefficient; in addition, allowing settling in such a time as 1864, before the first known production of commercial pasteurizers, could mean the unintended development of microbes in the milk²⁵. Thus, Prandtl produced a dairy centrifuge in order to expedite the process of separating the layers of milk into its heterogeneous state by order of density, a technique that would later evolve in the 20th century to be used in general medicine, mainly with regards to separating

cells from patient serum^{24,26}. Instances of this process with regards to Alport Syndrome will be detailed as this review advances chronologically, but the first known use of a centrifuge for analytical purposes was in 1925, with a machine produced by Theodor Svedberg that would later earn him the Nobel Prize²⁴.

The 20th Century and a Boom in Kidney Research

With the first half of the 20th century came vast swathes of new information, as communication between scientists in other parts of the world was becoming easier and information was becoming much more accessible. From further advancements in microscopy, centrifugation, and genetics, as well as updates to old studies with this improving technology, the amount of new and exciting milestones alone are almost too many to mention in this relatively brief review. Beginning with the reclassification of renal diseases into what we generally know them as today, a very important standardization for all future studies and a foundation for all kidney research to gain its bearings, the foundational paper came about in 1914 from scientists Theodor Fahl and Franz Volhard which they called *Die Bright'sche Nierenkrankheit, Klinik, Pathologie und Atlas*, and refined on the observations made by Bright by categorizing kidney diseases based on their mechanisms²². There are three such classifications: Nephrosis, which covers degenerative diseases of the kidney; Nephritides, which cover inflammatory diseases of the kidney; and chronic nephrosclerosis, which by happenstance is the characteristic nature of Alport Syndrome²². In addition to the aforementioned discovery of X-linked glomerular sclerosis in Alport Syndrome, which was incorrectly labelled hereditary nephritis by Alport himself in 1927²⁰, other advancements before 1950 include advancements in microscopy technology. For example, Thomas Addis' life work revolved around renal cell observation in patients, including autopsy examinations of renal cells and urine, where he noted the formation of "casts", cylindrical structures indicative of aggregation of blood cells or other macromolecules such as proteins, for the first time in papers

he published in 1931^{21,22}, which has subsequently become part of modern screening methods for quantifying the progression of chronic kidney diseases.

In the 1950s, wherein screening methods using urine microscopy were more fully understood and renal *autopsy* was a fully practiced feature of studying renal disease, the fact that no active glomerular monitoring methodology existed or was popularized yet prompted the next logical step, one which would become absolutely essential to any and all future treatments and clinical trials: renal *biopsy*, an active monitoring of the disease state. Given the recently published work of Addis, which details full progressions of dozens of patients from onset to post-mortem observation, as well as the nature of certain chronic diseases such as Alport Syndrome acting progressively, it quickly became essential to understand the entire progression of chronic kidney diseases, rather than the end state alone. With this in mind, there was an attempt before this decade to perform clinical biopsies of renal tissue; Nils Alwall, a Swedish renal pathologist, successfully gathered samples from 13 individuals using renal needles to remove tissue samples, but a patient's death caused him to cease and he ended up publishing in 1952, rather than when he was doing his research in 1944²¹. Fortunately, in the interim between Alwall's work and his publication, the famous Claus Brun continued successful needle biopsies and produced results along with Paul Iversen that were published in 1951²¹. A common theme among the technologies described so far, rapid development of biopsy techniques led to Robert Kark's innovation of using a cutting needle, the Vim Silverman design, rather than an aspiration needle to draw renal samples more efficiently for study in the year 1954²¹. Based upon the understanding of these renal biopsy techniques, the first real progress into *treating* kidney diseases, including Alport Syndrome, made its way into scientific discussion, and before any other advancements in machinery such as computers or drug development, there were two key solutions being developed to actively treat kidney disease and renal failure: dialysis, and kidney transplantation.

With regards to the former treatment, dialysis is certainly far less invasive than full transplantation, and ideally limits extraneous factors, such as individuals donating functioning kidneys. The procedure, in its most basic operating definition, is to “clean the blood” of the patient the requisite machine is attached to, and the initial version of said machine was invented in 1943 by Willem Kolff, a Dutch nephrologist moved by witnessing patients suffering end-stage renal failure²⁷. In particular, this machine was suited to treating acute kidney failure rather than disease, and only one of twelve initial treatments were temporarily successful, prompting Kolff to improve his design during the 1950s, after immigration to the US²⁷. It was there that Kolff’s work, in addition to the advent of Teflon tubing, would lead to the development of what is known today as hemodialysis by Dr. Belding Scribner, wherein an extraneous device, called a dialyzer, is used to directly clean blood from the patient’s bloodstream before re-entry via tubular shunts connected at the beginning of each regularly scheduled treatment^{27,28}. The first successful long-term treatment, qualified as more than one year of survival after beginning dialysis, came in 1960 with patient Clyde Shields. Clyde survived a further 11 years after renal failure due to this new continuous treatment and technology before dying of a myocardial infarction in 1971, a common theme with two other patients who began treatment after Shields and survived 28 and 14 years, respectively²⁸. To improve this initial version of long-term treatment (and while the aforementioned patients were undergoing theirs), the shunts were upgraded with the use of Teflon tips and thinner tubing, made of silicone elastomers, to make a more flexible shunt that was simultaneously less prone to blood clots^{27,28}. In the year 1962, an additional advancement in hemodialysis came in the form of the introduction of arteriovenous fistula procedures, utilizing a blood pump to remove blood from an easily accessible vein (such as a brachial vein), dialyze the blood, and reintroduce the blood into another part of the body to avoid issues with access to non-dialyzed blood with the use of shunts²⁷. Thus, the use of shunts has been phased out. While most hemodialysis was performed at dedicated clinics or hospital sectors during this time,

the sheer capacity of such centers was often a limiting factor for who could receive treatment once the fledgling treatment gained attention and before its influence spread to other parts of the country and the world^{28,29}. So, when one of Scribner's colleague's friend's daughters was in need of dialysis but turned away due to lack of capacity, Scribner et al. were prompted with creation of a hemodialysis machine for home usage, which they quickly developed, and machines of this nature were soon an extremely popular method of treatment throughout the latter half of the 1960s and onward, a now-standard practice in general²⁷⁻²⁹.

Around the same time, in 1959, another type of dialysis was first successfully used to treat renal failure by one Dr. Richard Ruben in San Francisco that aimed to avoid the use of mechanical machines entirely, instead relying on another portion of the patient's body to bear the responsibility of cleaning blood. This method would come to be called peritoneal dialysis (PD), so called for the use of a patient's own stomach lining, the peritoneum, to filter blood via usage of an extraneous dialysate solution^{27,28,30-32}. The ultimate goal of such a treatment was to enable ambulatory care and subsequent outpatient treatment so that patients could function in a normal capacity rather than receive inpatient care for dozens of hours a week, a staple of hemodialysis that still permeates today²⁸. While, like hemodialysis, attempts at PD began in the 1920s and 1930s, plagued by issues of sterility and inadequate materials, as well as non-standardized dialysates, the only fully standardized treatments would begin in the 1960s with a program headed by Fred Boer at the University of Washington^{30,31}. With regards to peritoneal dialysis, the introduction of a revolutionary catheter specifically designed to access the peritoneal region which would allow for easy access to the important biological treatment interface came in 1968 from the mind of Henry Tenckhoff, a colleague of Boer, who was the major proponent in developing the first standardized PD program after Boer's exit^{30,31}. This catheter was designed using a material based on silicone elastomers, the same kind used for the shunts developed for earlier hemodialysis treatments as well as many other procedures

involving catheters, even today^{28,30}. The first self-treatment using PD came in 1962 with an automatically-cycling machine developed by Boer's team for home use, which was quite successful, and which led to later improvements in the 1970s, including the advent of a self-sterilizing system in 1972^{27,28}. Today, these particular types of dialysis are the most prevalent treatments for patients whose kidneys are failing whilst on a waitlist to receive a potentially long-term solution: a kidney transplant.

Kidney Transplantation and Modern Immunosuppression

Much like hemodialysis and peritoneal dialysis, kidney transplantation was a burgeoning wealth of experimental potential exploited heavily in the time between the Great War and World War II. While kidney transplantation had been experimented with for decades prior, the plausibility of performing human-to-human transplants was only realized in 1936, when the first human-to-human transplant was attempted³³. This kidney, along with one in a documented attempted transplant in 1939 were both from deceased donors and failed less than a week after their insertion; unsuccessful procedures^{21,27,33,34}. It was clear that there was a fundamental issue regarding *why* these kidneys were failing, and the research this prompted started in the throes of World War II with results from researchers such as Sir Peter Medawar eventually uncovering the issue: the body's own reaction to foreign objects, such as a foreign organ. The crux of the issue in this case was that the recognition of an exogenous kidney, the donor's, caused the body to produce an immune response very similar to those presented for any other condition or disease, and the antibodies produced (whose mechanisms are only now being elucidated!) would have the effect of *destroying* the new kidney, even though it was initially "healthy". This term is now known as "rejection", called so because of the recipient's body's refusal to retain this "invader", and this phenomenon was one of the largest barriers to knock down in order to achieve successful long-term transplant treatments for patients with kidney failure. Without immunosuppression, the longest transplant-to-death timeline up until 1952 was a couple of days at most, until one attempt using a deceased mother's organ in a transplantation with her son led

to a survival period of 22 days and into the year 1953, lending additional credence to the idea that genetic relatives might tolerate transplantation better^{27,31,32,34}. In 1954, a transplant between two identical twins produced promising results, as the transplanted kidney would survive eight years without any form of immunosuppression^{32,33,34}. As the kidney was genetically identical, the clear differentiator became apparent; the immune system of the recipient was going to reject a new kidney unless it was genetically identical, *or the recipient's immune system was suppressed*. At this point, research funding immunotherapies shifted into the spotlight, and by 1962 the first commercial immunosuppressant drug, known as azathioprine, would be developed^{33,34,35}. Thus, the first long-term, genetically unrelated kidney transplant procedure would produce a kidney that survived for 21 months in the recipient's body, a leap of more than a year from the previous best result. This catapulted immunosuppressant research even further into relevance, and in 1972 one Jean Borel introduced to the scene his discoveries on the immunosuppressive qualities of a drug called cyclosporin, which more actively worked to suppress patients' immune systems^{35,36}. Cyclosporin was approved by the FDA one year later and became recognized at the time as the most successful immunosuppressant drug, though this would not stop continuing research on drug development. Both azathioprine and cyclosporine were (and still are) known to produce many side effects, including hypertension and nausea in addition to tremors and numbness in the extremities^{12,33,34,35}. Today, additional drugs such as tacrolimus (Prograf), introduced in 1989 and approved by the FDA in 1994, have become a common part of some transplant patients' regimens due to the broader range of treatment and dosages it offers, although the aforementioned azathioprine and cyclosporine are still prevalent^{12,33,34,35}.

Today's Progress and Where It's Headed

When genome sequencing and genetic counselling services entered the mainstream, heritable diseases like Alport Syndrome gained some much-needed data and relevance, prompting the creation of a dedicated database for all known mutations. Through genetic

sequencing, early observations by Alport himself were finally validated; through case studies of patients with Alport Syndrome, three specific genes would stand out as the clear identifiers for Alport Syndrome: COL4A3, COL4A4, and COL4A5³⁻¹². COL4A3 and COL4A4 are located on chromosome 2 in humans, while COL4A5 is located on the X chromosome, and the latter is the gene Alport had observed so many decades before, wholly responsible for the X-linked inheritance pattern he'd observed. These three genes are responsible for the formation of one specific structural protein found in the glomerular membrane, as well as in the eyes and the ears called type IV collagen, and at this one specific variant that relies on the association of one copy of each gene. For this reason, scientists call this version of type IV collagen a "matrix", denoted "IV (345)". Other matrices of other subunits or types of collagen exist, but the aforementioned matrix is specifically responsible for Alport Syndrome. Each of these genes encodes one subunit of the overall collagen structure, and contains code for a collagenous region, repeating a glycine-proline-proline amino acid residue pattern, until encoding a more globular structure at the C-terminal end, named the non-collagenous (NC) region. To form a IV (345) matrix, one copy of the 3, 4, and 5 subunits each non-covalently associate and allow their respective collagenous regions to also associate, thus forming the structural protein in its wild-type configuration. When an issue with any of the subunit genes prevents the NC region from being translated, or otherwise disrupts the structure of either of these regions (usually the collagenous region), the function of the structural protein is compromised, leading to the phenotypes associated with Alport Syndrome. Modern therapies and new proposals related to Alport Syndrome stem from this general basis, and since X-linked Alport Syndrome is most common, most therapies are directly related to COL4A5.

New therapies for hereditary diseases are developing rapidly; since the 20th century, advances in fields of protein engineering, genetics, biotechnology, and more access to scientific resources, most notably with the advent of the Internet, have all been promoters of such rapid

growth in resource allocation to genetic diseases that this review paper would have trouble describing each and every single one. Therefore, to cover a small sample of the newly proposed therapies, this paper will be limited to two separate measures the scientific community is currently undertaking with regards to Alport Syndrome: accurate modelling, and a special technique revolving around RNA splicing called “exon skipping”. With regards to the former, an advancement in monitoring techniques such as diet control and efficient CRISPR/Cas9-mediated DNA repair has allowed researchers of Hashikami et al. to accurately model disease progression in an orthologous fashion to human Alport Syndrome using mouse models³⁷. Mice possess orthologous genes for collagen production that share conserved function to humans; this would make sense considering that mice are around 87% genetically identical to humans³⁷⁻⁴⁰. By harvesting wild-type zygotes and exposing the single cell to a Cas9 system introducing a mutation in a mouse’s orthologous COL4A5, then reintroducing the zygote to a pseudopregnant mouse, the authors could alter mice to express Alport Syndrome phenotypes, and in contrast to previous studies, these mice produced analogous disease progression profiles relative to both symptom development and timeline as documented human cases. This research became the basis for another study, by Yamamura et al., dealing with ameliorating the specific R471X (a truncation mutation) mice that Hashikami et al. developed.

The study by Yamamura et al. noted that Hashikami et al. had created a superb template, a sort of base system, for studies like theirs to be conducted efficiently, and without necessitating the use of human patients or non-organismal cell lines (*in vitro* studies) to create meaningful results⁴¹. In order to utilize the potential of the mouse studies, this study used mice generated with the same experiment parameters as the previous study, but experimental mice were additionally exposed to a mechanism designed to cause the ribosome to *ignore* the specific codon encoding the “STOP” signal. This allowed a slightly truncated, but functional version of the collagenous region to be produced⁴¹. Notably, this methodology would produce

functional type IV collagen in individuals who would not otherwise do so. The results of the study showed great promise, and a pre-clinical trial in humans is now being developed. With such advances in a field that knew no treatments less than one hundred years ago, the field to Alport's contemporaries is likely unrecognizable, but today's best stand on the shoulders of giants.

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