This talk is themed on a movie, Back to the Future, which was a popular series of movies in the mid '80s and I will walk you through the story line. So Steven Spielberg presented Back to the Future in 1985, spent more than a billion dollar movie, set of movie series and the theme was that with the help of a whacky scientist a young teen travels back to 1955 in a DeLorean turned time machine. Once there he meets his parents but things go out of whack and he must ensure that they are corrected or else we will never come to exist himself.

So in a twist on this theme I'm going to present the nephrotic syndrome story. The theme is a little bit twisted too with the help of a whacky scientist doctor, which is me. We are going to travel back in time with a PowerPoint turned time machine to understand the origins of this disease. Once there we will understand how things go out of whack to cause proteinuria / nephrotic syndrome and what must be done by current and future advances to ensure that it is corrected or else.

So this is kind of back, these are the stalwarts of nephrotic syndrome and it's diagnostics and how we came to know a lot about this particular syndrome. back in the 1950s and '60s. Dr. Renee Habib was a prominent pediatric nephrologist in Paris, Dr. Robert Vemier was in Minneapolis, I did my fellowship with him and I had enough fortune to do work with Dr. Barratt when I was in London become coming to America. This is the present and the future of nephrotic syndrome, I'll be talking about some of their work as we go back.
But let's start from really, really back, back to the time of Hippocrates. It seems like he was the first guy to discover nephrotic syndrome, that's when he identified when bubbles sit along the surface of the urine it indicates the disease of the kidneys and the complaint will be protracted.

So what is nephrotic syndrome? Nephrotic syndrome is a combination of findings all of which need to be there to call it the full blown nephrotic syndrome. That includes proteinuria which should be more than 2 grams per 1.73 meters sq per day, hypoalbuminemia which should be an albumin level of less than 2 grams per deciliter, edema as well as hyperlipidemia, which is typically indicated by a cholesterol level of more than 200. Some of the causes of nephrotic syndrome can lead to end stage renal failure and that's why it's important to know as much as we can about this particular disease.

So what are the common causes of nephrotic syndrome? The common causes are minimal change nephrotic syndrome and FSGS, a Focal Segmental Glomerulosclerosis. The FSGS is the entity that common leads to end stage renal disease and this is the one that we mostly worry about when we see these children. Less commonly seen causes of nephrotic syndrome in childhood are membranous, membranoproliferative and secondary to other diseases.

For this particular talk I'm going to use NS / FSGS in a single phrase and we are just beginning to know about the etiology of FSGS, It has been largely unknown over all of these 2500 years although a variety of factors have been implicated. So for this talk I'm gong to share the accumulated knowledge of nephrotic syndrome with a particular focus on genetics over the last 2500 years, identify key hereditary nephrotic syndrome phenotypes, some of the nephrotic syndrome
NEPHROTIC SYNDROME: BACK TO THE FUTURE,
ABBAY VATS, MD

So why do we even want to know about genes for nephrotic syndrome or FSGS? It's important because the unraveling of molecular mechanisms of glomerular permselectivity, they can help us identify the prognostic factors and those that possibly identify new therapeutic options. As we go along we'll see how each of these has played a role over the last few years. Indeed, prior to 1950s therapeutic attempts to cure nephrotic syndrome included various antimicrobials, hot cages, purges, Southey's tubes, mercurial diuretics and even iatrogenic infection with measles and malaria. Those were pretty horrific ways to treat nephrotic syndrome back then. We have come a long way since then.

So how do we even study about the genes that can cause nephrotic syndrome? There are two classical ways of doing it. One is forward genetics, the other is reverse genetics. Forward genetics is when you identify the renal diseases and you identify the genes based on the patients that you identify. Reverse genetics is when you identify the genes first and then you identify the syndromes associated with those genes. For identification of genes associated with nephrotic syndrome both of these approaches have been applied. In addition to the classical approaches new ways of studying this particular disease are coming along including studies of gene environment interactions or GEI studies where various types of foods or lack of certain elements or infections can play a role in the etiopathogenesis of nephrotic syndrome. So all that we have known about nephrotic syndrome for
over 2500 years we I think only know very little about it, just the tip of the iceberg, a lot remains to be discovered and identified.

I'm going to walk you through some of the genes and the phenotypes associated with it. We have over the years found that several types of nephrotic syndrome can be associated with single gene effects or they can be polygenic or they can be due to gene environment interactions. So over the years especially the last 10 to 15 years we have come to identify that podocyte is the culprit behind most of the genes associated with nephrotic syndrome and there is a particular term that has been coined over the last 10 years or so, it's called podocytopathy. So it's perturbations in podocyte genetics and architecture and interactions with its surroundings that lead to practically all of the causes of nephrotic syndrome that we know so far.

This is the gross podocyte anatomy of the architectural details. These are the foot processes, triangular foot processes that sit on the top of GBM or the glomerular based membrane which is the filtration barrier and across the filtration barrier is the endothelial fenestrated surface. The blood flows through this capillary and the urine passes through the fenestrated pores through the GBM and across the slick membranes, or slick pores. So when some of the genes get altered or perturbed in nephrotic syndrome we see that some of this architecture gets significantly altered.

I'm going to touch base upon some of the key genes of nephrotic syndrome and their key features now. As we all know, there are certain terms that we need to deal with: the genotype which is the mutation that's identified in a gene and the phenotype which is the description of the consequences
of the gene mutation or the syndrome. The gene disorders can be broadly classified into the recessive or dominant and the recessive can have penetrance which is full or which can have onset which is fairly early, and the recessive ones are quite rare. The dominant types of nephrotic syndrome genes have a penetrance that can be sometimes incomplete. The onset is usually late adult - late childhood to adulthood and the frequency though rare is less so than autosomal recessive genes.

I'm going to touch base on a couple of key genes both of autosomal recessive type and autosomal dominant type. The two main types, the two main autosomal recessive genes associated with nephrotic syndrome are NPHS1 and 2. They were labeled because they were identified in batch sequence almost 15 years ago. The first gene to be identified with nephrotic syndrome was NPHS1. It was identified by Dr. Carl Tryggvason from Finland. This gene is associated with a particularly bad type of nephrotic syndrome called congenital nephrotic syndrome or Finnish type or CNF and there are two major sites of mutations, Finn major and Finn minor. And interestingly some of these mutations are also seen in the descendants of Finnish and Scandinavian populations who have settled down in the University of Minnesota and in the Pennsylvania area. These children are typically steroidal drug resistant and they are born (inaudible) their kidneys are full of very dissolved glomeruli and nothing can fix their problem. In fact the only way we can fix them is to remove their kidneys. So it's a pretty drastic way of treating nephrotic syndrome, you remove kidneys, there is no proteinuria and there is no problem. We can't do that for many other organs. So what we typically do is remove their kidneys, put them on dialysis and grow them to about 10 kilos in weight and then transplant them.
This was the very first gene to be identified for nephrotic syndrome, the Nephrin is a protein that is located on the edges of these foot processes and this is a special staining called immunoelectron microscopy and it seems if you stretch a little bit of your imagination that these two proteins talk to each other. In fact subsequent studies have shown that Nephrin forms almost a zipper like structure which when perturbed leads to unzipping of these slick pores.

A few years after the identification of NPHS1 the second gene for nephrotic syndrome was identified. It was called Podocin or NPHS2 by Corinne Antignac's group. She used to work with Dr. Renee Habib. And over the last 5 to 7 years we have identified this gene to be the most important gene for childhood onset nephrotic syndrome and these studies have been replicated in many centers and many populations across the world. This now has been found to cause almost 50 to 55% of familiar FSGS cases and they can be associated with mutations in other genes to call digenic inheritance of both NPHS1, NPHS2 and even other genes. One particular variant there the R229Q is the most common one and it codes for late onset FSGS and complete steroid resistance. Recently a gene has been associated - identified for steroid sensitive nephrotic syndrome, it's called EMP2 and this is just hot off the press by a group from Michigan from Dr. Hildebrandt's group.

So those were some of the most common genes that we would see in childhood but as we started to see older patients in our clinics we tended to see even autosomal dominant genes that can cause this particular problem. Some of the genes that I'll be talking about are CD2AP, ACTN4, INF2 and WT1.
CD2AP or CD2 associated protein was a classical gene that was identified by reverse genetics. A group of workers were trying to knock out a mouse for looking at some of the immunological properties of this particular gene. It seems like the mice died fairly early but when they studied the immunological problems they were trying to identify, but the mice had features that were fairly typical of congenital nephrotic syndrome with (inaudible) that I had just shown you for congenital nephrotic syndrome or the Finnish type. So instead of developing a mouse with immunological problems they developed a mouse with nephrotic syndrome. Subsequent studies have been done to assure that these mutations have been associated with human disease also. Interestingly this protein, the homologous protein for CD2AP is located on chromosome 6 which is as you know the main location for HLA markers. So over the years it had been found that there were several HLA markers that were associated with a tendency to inherit nephrotic syndrome and this might be the connection.

Dr. Martin Pollock's group from Boston identified the first autosomal dominant nephrotic syndrome gene, it's called alpha-actinin 4, had been collaborating with him at that time and this particular protein belongs to a member of several proteins that are of the spectrin family and bind and crosslink actin and are important in organization of the cytoskeleton and in cell adhesion. This particular gene causes nephrotic syndrome that is typically seen in adults and has incomplete penetrance and can be mild or very asymptomatic. And these patients can just present with asymptomatic proteinuria.
Another gene that has been associated with autosomal dominant inheritance is called INF2 or inverted formin 2 and they - many of these mutations have been formed to cluster in one particular exon that is the exon 4. And it is one of the proteins that promotes nucleation of actin, which again is important in mentoring the cell cytoskeleton.

There is a particularly interesting autosomal dominant gene that is associated with nephrotic syndrome which has connection with actually this hospital. This particular gene is called Wilms' tumor 1 or WT-1. It has been associated with Denys Drash syndrome or DDS and Frasier syndrome. The Denys Drash syndrome has congenital early onset DMS as its main finding. It's associated with Wilms' tumor and missense mutations in one particular set of exons which is exon 89. The Frasier syndrome is also associated with Wilms tumor 1 gene mutations and it's associated again with intersex, FSGS and gonadal blastoma and there are specific intronic mutations that are associated with this particular syndrome.

I don’t know if many of you know, Dr. Drash was one of our own faculty in the Department of Endocrinology for a number of years who identified the syndrome. And this is how a diffuse mesangial sclerosis picture would look like in some of these patients. And Dr. Drash died 2009 after serving in this hospital for a large number of years and we are all indebted to him for identifying this particular syndrome and many of the problems associated with the childhood endocrine issues.

So I've just given you a catalog of a large number of genes that can be associated with nephrotic syndrome. Where does this all take us? This brings us back to the podocyte and the foot process
GBM interactions. There is a cartoon that shows some of these proteins in connection with each other. This is the very first protein which was identified, nephrin, it talks or interacts with a nephrin of the adjacent foot process and forms a zipper-like structure. The nephrin is connected to CD2AP which is connected to the acting cytoskeleton which is maintained by ACTN4 under the influence of WT1 and other factors. And they all interact with GBM or glomerular based membrane.

So as you can see, if anything goes wrong with any of these proteins the whole fancy architecture or fancy house can fall apart. That's what actually happens. So this is how a normal podocyte structure would look like. This is what happens when any other gene gets disturbed that is associated with nephrotic syndrome. So the final consequence of disturbed, or undisturbing or mutation of any of those genes is a loss of podocyte architecture.

Now we know besides the genes that I just mentioned there are at least 25 genes that have been associated with nephrotic syndrome and the list keeps on growing. Lots of them are autosomal recessive. I have touched base with on a few of them, nephrin, podocin, CD2AP, WT1, etc. Interestingly over the last couple of years an increasing association of non-DNA targets has also come along with nephrotic syndrome etiopathogenesis. And one of them was micro RNA 193A and over the last year or so there has been an explosion of interest in this particular molecule and I'll show you some of our data on this particular target in a little bit. So all those genes were just a few of the proteins that are found in a podocyte. The story gets much more complex and much more complicated if we try to look at a large number of proteins that can be expressed in the foot processes. I'll focus on some of them that are of particular interest to us in our studies which I'll
present next. So just look at some of these proteins that are interacting with some of the key proteins that are known to be mutated in nephrotic syndrome. These are alpha actinin, utrophin, catenin and actin.

So we started our studies soon after I joined here and some of our initial observations led to identification of new locations for these genes which are on chromosome 19, 11, 9 and 13. It all started with a family that I saw with Dr. Ellis in a clinic with a medical student at that time, Alex Nark. This particular family continues to come to our clinic even now, and what I found with some of our fellows and our residents we typically bring up this paper. So this is an interesting family, this is an Afro-American family which has autosomal dominant nephrotic syndrome and unlike the autosomal dominant phenotype that is typically seen with other genes that Dr. Pollok and others have identified these children start having problems really, really early, almost like congenital nephrotic syndrome. And they are nephrotic and very edematous within 2 to 3 months of age and they remain proteinuric throughout their lives and don't go into renal failure until fairly late. And this particular grand old man who Dr. Ellis used to see went into renal failure in his 50s and we saw some of these patients in our clinic and some of our nurses actually one of them was involved with their care when we used to see them, and now we have some of these kids having kids and they have nephrotic syndrome so we are actually seeing them in our clinic and they get admitted to our hospital even now.

So after that we identified a fairly large family, again an Afro-American family, again seeing Dr. Ellis for quite some time, and Dr. Moritz and Ron Jaffe helped us identify some of the other features
of this particular family. All of these studies were done in collaboration with Dr. Paul Ferrell in the Dept. of Human Genetics. This particular family was very large, almost 100 member family. I'm just showing you a very small portion of it and this phenotype also presented in an autosomal dominant manner and this was our patient who had initially presented to us with just an asymptomatic proteinuria finding that was discovered during a routine school physical exam. We evaluated him, did kidney biopsy, our own Jeffrey was the pathologist at that time and we identified features which were quite typical of nephrotic syndrome as well as certain vascular features which were very hypertrophic vessels and he was quite hypertensive even at a young age of 12 years.

So this particular patient went into renal failure within a couple of years of seeing us starting from very mild asymptomatic proteinuria to complete renal failure within 2 years. We had to transplant him, but as a part of taking care of him we got to take the family history and it turned out that many other family members had very mild proteinuria, all were hypertensive and his sister who was younger had practically nothing when we saw her but when we genotyped she had the presence of high risk genotype for her. So to cut a long story short this particular girl went to - joined the Army, went to Iraq and then one fine day came back to see us in the clinic and it turned out that she had developed significant proteinuria. So we had identified her particular problem before it even got started by looking and analyzing her genotype.

So we did further studies on this particular family, found a fairly high LOD score which is a P value that links a particular genomic region to a trait and normally the P values for usual studies are .001 or .05, here we had a 10 risk to power -6 probability value, so it was very, very likely to be
associated in this particular region of the chromosome. This particular chromosomal region was explored by us and it had several interesting genes including actin like 7A, actin line 7B, and catenin like molecules. These are particular molecules I just showed you in that cartoon so we really went after these particular genes initially to identify the causative mutation. Then we started from top to bottom and started to sequence the head count of this region.

It turned out that this particular region was fairly big and we couldn't - we sequenced almost 80 to 90% of these genes but didn't find a particular mutation, never sequenced the intronic regions. So we kind of left it at that. And that's when the future comes in, our fellow couple of years ago started looking at this particular region again with newer techniques which had come along over the last 5 years or so which were based on next generation sequencing. So we decided to target this particular region by genome sequencing and whole exome sequencing including targeted deep sequencing of that particular region.

This is just a cartoon showing all the various steps involved in this particular project. And we were encouraged by findings of other people who had identified new genes just by following this particular approach. And this particular gene was identified by whole exome sequencing last year and an analysis of our data has honed down this particular region to about 4 or 5 interesting snips or mutations that we are now working with some of my colleagues to identify their functional sequence.
So again the podocyte is the center of the universe for nephrotic syndrome. This is the very normal glomerular architecture with nice little pyramid transverse foot processes and they all go haywire once anything goes wrong, any of the gene goes wrong, the whole house of cards falls apart. But although we have built the house of cards just to identify the genes and to know a little bit more about this particular problem we have also identified ways of cheating particular problem in new manners and new therapies have arisen. And also we have learned how even older existing therapies work. So we have identified now over the last few years that corticosteroids may ameliorate the kidney injury by increasing actin polymerization through GDPases and calcineurin inhibitors like Prograf or Cyclosporin may work through TRPC6. So this is where the TRPC6 is and actin skeleton is stabilized by corticosteroids. So we have used these therapies for a long time without knowing actually how these therapies work, but with the identification of these new genes and new mechanisms we are actually learning why they even work in some of the patients.

So now we know about all of these genes they can help us identify how to treat some of these patients better. How do we start approaching their diagnostics? So it all starts with a very good history and physical exam in the clinic. We can identify isolated disease or we should try to look for various syndromes that can be associated with them like Denys Drash syndrome, Nail Patella syndrome and so on. We can also identify when the disease starts in early onset or late childhood. And the earlier the onset of the disease the more likely is a mutation going to be found in one of these, in one of the genes that I just mentioned about. Later onset diseases are less likely to have identification of a particular mutation.
There are now companies that are doing these particular tests but unfortunately these do 1, 2, or 3 or 4 genes at a time. As I just mentioned a little bit ago we are - we are at a stage that more than 25 plus genes have been identified. So how do we even try to approach such a big gamut of genes and the large number of mutations that can be associated with them? So newer technologies are coming along including whole-exome sequencing that I mentioned and even targeted sequencing approach, some of which we undertook.

And lately a particularly interesting approach has been published just a few months ago from Hildebrandt's group where they did massive multiplexing and amplified and sequenced almost 500 amplicons belonging to 21 genes all in one go. So instead of sending 1 or 2 genes at a time for sequencing all 22, 21 genes could be sequenced and the results were available in less than 3 weeks, and the costs were much less than what (inaudible) charges us and it could detect mutation in 1/3 of the cases that they were looking for. So this is a very interesting approach to identification of genetic causes of nephrotic syndrome.

So I'm going to change gears now and just for the last few minutes I'm going talk about some of the treatment approaches and how some of the genetics and some of the newer knowledge that we are accumulating help us decide some of the treatment options.

Traditionally nephrotic syndrome has been treated with steroids, in fact that's how we classify nephrotic syndrome. It's started classified into steroid sensitive or steroid resistant nephrotic syndrome whereby the steroids are given for a period of almost 2 to 3 months initially and if they
respond within the first 4 to 6 weeks we call them steroid responsive. And if they don't then we call
them steroid nonresponsive or resistant. Within steroid responsive patients you can have frequent
relaxers or steroid dependent patients. Those are fairly well defined outcomes of steroid response.

Once a patient fails steroid therapy we go onto the next layer of treatment options including
Cyclophosphamide, Chlorambucil which are given for a period of no more than 3 months and
approximately 25% of patients who would show some response to steroids but would begin to
develop steroid frequent lapsing type of a pattern would respond to Cyclophosphamide or
Chlorambucil therapy.

Once they fail this particular set of therapy then we go on to the unknown, which is trying
Tacrolimus, CellCept, a combination of that or a combination of this, this and that and we almost
give these types of treatments forever. So we see of the patients that were treated with Tacrolimus
at least a third of them would respond to it, a third would be partially responsive and a third would
not respond at all.

So we are left with a significant proportion of patients on whom nothing works so that's where some
of the newer types of therapies have come along including use of some monoclonal antibodies over
the last 5 to 7 years. One particular monoclonal antibody is Rituximab. This is a chimeric antibody
directed against CD20 cell surface receptors of B cells and it was initially approved for non-
Hodgkin's lymphoma, but it has been used for SLE and rheumatoid arthritis and low and behold it
has been found to be useful for some of our very difficult cases with the current minimal change
disease or idiopathic FSGS. We have one particular patient who is actually on the floor right now who is getting this particular therapy.

Interestingly, this particular therapy seems to work in patients who respond to steroid at some stage. So this was a study just published in June, 2014. This is the first multicenter double blind randomized placebo-controlled study for this particular molecule. Here 52 patients underwent randomization between 2008 and 2010 in European centers, 48 were assigned to intervention of which 24 were given Rituximab and 24 were given placebo. The median relapse-free period in the treated group was close to a year, 267 days versus less than half of that in the non-treated group. So this is a very powerful treatment option that has emerged over the last 5 to 7 years.

Unfortunately some patients don't respond to CD20 blockade. Fortunately to our rescue a new therapy has just emerged. This was published last year in December 2013 from Peter Mundel's group, Abatacept in B7-1 Positive Proteinuric Kidney Diseases. So there is another particular molecule called CD18 whose blockade seems to help patients who do not respond to Rituximab therapy. This is characteristics of various patients, they had published their brief communication on these 5 patients, 4 of them had relapsing focal segmental glomerulosclerosis after transplant, 1 of them had it in the native kidney and nothing worked for them, Plasmapheresis, steroids, Tacrolimus, Rituximab, everything failed. They were given either a single dose of this particular agent or 2 doses or multiple doses and it seemed to put them into complete or near complete remission. So this has really exploded onto the scene over the last year. Another particular CD20 antibody is also coming
along, it's called Ofatumumab and this is also useful for Rituximab resistant nephrotic syndrome patients.

So it's been a few years since any known markers have been associated with nephrotic syndrome or FSGS except for presence of protein in the urine, that's how we diagnose and follow these patients. But a lot of search has been made for other biomarkers that can help us predict and guide therapy and lately 2 particular markers are showing particular promise. One of them is urinary CD80 or B7-1, which is increased in urine of patients with minimal change nephrotic syndrome. Patients and not usually FSGS patients and here this particular therapy seems to be useful. Another particular marker is suPAR which seems to be increased in the blood of FSGS patients and there almost nothing seems to work.

Over the last couple of years an interesting molecule has also come onto the scene which includes micro RNAs. This particular molecule is a small noncoding RNA of about 22 nucleotides each and it regulates the posttranscriptional gene silencing by binding to the untranslated region of the genes. And its expression pattern can indicate the pathophysiological status of various tissue and is a change in various disease states.

A lot of work is being done in identifying micro RNAs associated with various disease conditions, various cancers, various other problems and just over the last year almost 10, 12 papers have appeared where different micro RNAs have been associated with nephrotic syndrome and FSGS.
Our own studies have taken us also in this direction in collaboration with Dr. Jacquie Ho and she has been particularly setting a particular micro RNA called micro RNA 30a-5p which is involved at various stages of kidney development. She postulated that it may be dysregulated in nephrotic syndrome FSGS as many of the other genes that are associated with genome development are. So we did a preliminary study in 6 FSGS vs. controls and we formed 5-20X upregulation in this particular micro RNA in the urines of these patients. We have gone on to develop a new way of actually amplifying and detecting this particular type of molecule and we are calling it CHAMP and just recently filed an invention disclosure. These are some of my team members.

So the future has brought us into very interesting directions. We can now talk about epigenetics, pharmacogenetics including new ways of sequencing our patients' DNA or even RNA and we can make genotype, phenotype correlations and probably predict who will go to ESRD or who will respond to therapy or even what type of therapy to choose. So it might come to something like this, you can take a person's DNA, blood, urine, profile them and come up with a very tailored therapy with that particular patient's name actually imprinted on that particular treatment.

So just to conclude, nephrotic syndrome / FSGS are related disorders with widely divergent renal outcomes and they are a strong genetic component, they have multiple modes of inheritance. A good history and physical exam are key to identifying many of these syndromes and underlying pathophysiology. And diagnostics should be based on some of these very basic techniques and prospective treatment and therapeutic options can be based on the new genetic information and biomarker information.
So this concludes our story about the nephrotic syndrome. The cast for the story was podocyte, which represented Michael J. Fox, albumen which represented Leah Thompson who was saved by Michael J. Fox in going back to the future. So if we fix podocytes we'll fix albuminuria.

These are some of my crew members. I'm highly indebted to them for all their help and cooperation over the years. Alice was one of our first students, Dr. Alice everybody knows. Dr. Ferrell was my mentor and helped in my renal studies with classical linkage analysis. Dr. David Feingold and Dr. Ferrell have always worked together. Mary Jo helped me identify a lot of these patients, take care of them. Mike Morris, Dr. Ron Jaffe helped us with clinical assessment of many of these patients. My research associate Isba Chandra has been with me for almost as long as I have been. Some of my collaborators in the School of Engineering we have been working with them to develop new ways of detecting the biomarkers that we have been identifying. A fellow who worked on the novel exome sequencing project, Dr. Ho, for the micro RNA, Kathryn and Dr. Ho and Chandra developed new CHAMP technology and have been working with Dr. Agnes to develop some of the functional genomic assays for some of the variants that we are finding. I'd like to thank you for your attention.