THE NEPHCURE FOUNDATION

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RESEARCH UPDATE

The following is an overview of the research being conducted worldwide that is relevant to the NephCure Community. Please note that this is an analysis of current research presented in the field and is not specifically endorsed by NephCure.

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12/2009

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Working together to find answers: Nephrotic Syndrome Rare Diseases Clinical Research Consortium (NEPTUNE)

Minimal change disease (MCD) and focal segmental glomerulosclerosis (FSGS) generate an enormous individual and societal financial burden, accounting for approximately 12% of prevalent end stage renal disease (ESRD) cases (2005) at an annual cost in the US of more than $3 billion. However, the clinical classification of these diseases is widely believed to be inadequate by the scientific community and we still do not have a comprehensive molecular understanding. Given our poor understanding of MCD and FSGS biology, it is not surprising that the available therapies are imperfect. The therapies lack a clear biological basis, and as many families have experienced, they are often not beneficial, and in fact may be significantly toxic. Given these observations, it is essential that research be conducted that address these serious obstacles to effectively caring for patients.

In response to a request for applications by the National Institutes of Health, Office of Rare Diseases (NIH, ORD) for the creation of Rare Disease Clinical Research Consortia, the University of Michigan in association with a number of affiliated universities joined together with The NephCure Foundation in collaboration towards the establishment of a Nephrotic Syndrome (NS) Rare Diseases Clinical Research Consortium. A grant application was submitted for a multidisciplinary research and educational platform, to bring together clinical and translational scientists, and increase our knowledge about Glomerular diseases such as FSGS, and MCD. Through the efforts of Drs. Larry Holzman and Matthias Kretzler and the NephCure constituency, many of whom contacted congressional leadership for support; in September we received the stunning news that NEPTUNE was awarded a $6.25million grant from the Office of Rare Disease. This combined with a $2 million commitment from NephCure and a $2 million in-kind commitment from the University of Michigan combines for a $10 million five year study.

The importance of this program lies in the fact that it allows several major barriers in Glomerular research to be overcome to effectively conduct research and interventional studies. Most importantly this provides the infrastructure for continued collaboration of researchers in this field which will advance the care of MCD and FSGS patients internationally. The specific aims of the Consortium are as follows:

1. Establish an infrastructure to efficiently conduct clinical and translational research in nephrotic syndrome (NS).
2. Identify and characterize new biomarkers (used for the diagnosis of Glomerular diseases) and/or potential therapeutic targets for NS.
3. Conduct two clinical studies in NS.
4. Develop and implement a postdoctoral training program that is suitable and appropriate for training of individuals with MD/PhD to become skilled in conducting research in rare kidney disease.
5. Develop multimedia lay and physician educational resources on NS in collaboration with Nephcure (http://www.nephcure.org), and Halpin (http://www.halpinfoundation.org)
6. Develop and maintain a high quality, secured and user friendly repository of clinical data and biospecimens (a biobank) of NS patients for sharing among researchers nationally and worldwide through the web thus further stimulating research into these diseases internationally.

What's in *your* urine? The search for disease markers in Nephrotic Syndrome

An estimated 26 million American adults are believed to have some degree of impaired kidney function and by all accounts kidney disease is on the rise. While many of these diseases are treatable, the treatments are disease specific and a diagnosis is required. To make an accurate diagnosis, a kidney biopsy, an invasive procedure removing a small piece of kidney tissue for analysis, is required. Due to the risk involved, some patients may not receive a biopsy, thus delaying accurate diagnosis and treatment.

A great deal of effort has been made in search of noninvasive tests for early identification and classification of the type of kidney disease, to predict the severity and outcome as well as the patients' response to treatment. A major goal has been to identify biomarkers (proteins and molecules) that can be measured inexpensively for early diagnosis of disease in patients.

Proteinuria (leakage of proteins in the urine, especially the protein albumin) is characteristic of glomerular diseases which affect the filtering mechanism of the kidney, such as FSGS. Proteinuria is associated with progression to kidney failure. In an effort to examine the predictive value of other molecules that may better correlate with disease, Bakoush and colleagues have examined IgG, a protein normally found in the blood, which like albumin, also leaks into the urine in glomerular disease. Dr. Bakoush has found that the level of IgG in the urine was a predictor of kidney survival and that an increase of IgG above a certain level was able to predict 88% of cases that progressed to end stage kidney disease (ESKD) [1]. While this work holds the potential for determining the patient's prognosis, other researchers are taking a different approach to find markers allowing diagnosis of the various kidney disorders.

In one such study presented at the American Society of Nephrology conference, Dr. Jeroen Deegens and colleagues evaluated urinary exosomes, small, intracellular vesicles released from the filtering cells of the kidneys and excreted into the urine. Exosomes contain many proteins that may serve as urinary biomarkers in kidney disease. To meet the goal of finding biomarkers, Dr. Deegens continues to improve on laboratory methods to find proteins in exosomes from urine with nephrotic range protein loss[2]. The challenge has been the

development of ways to detect the small amounts of proteins that may be significant in the disease process.

While Dr. Deegens is in search of unknown biomarkers, Dr. Bennett and colleagues have used previously discovered markers to distinguish between two clinically similar diseases, FSGS and minimal change disease (MCD). FSGS and MCD have similar clinical presentations but much different prognoses. Dr. Bennett has evaluated two urinary biomarkers, NGAL and MMP-7, measured in the urine of patients with FSGS and MCD then compared to healthy individuals. The researchers found that NGAL levels were significantly higher in FSGS and MCD patients when compared to healthy individuals and 10-fold higher in patients diagnosed with FSGS. Furthermore, MMP-7 levels were significantly higher in patients with MCD but a significant difference was not found when comparing MMP-7 in patients with FSGS. Elevations in NGAL and MMP-7 may be a useful tool in the future in the diagnosis of FSGS and MCD but the study must be repeated in a larger group of patients before they can be used to assist in earlier diagnosis and individualized treatment[3].

The importance of this research goes beyond the need for earlier diagnosis of kidney disease; it may also affect the treatment options available for patients as well. Currently treatment decisions are based on measurements of kidney function which are inexact, or on repeat kidney biopsies which are invasive and carry risk. Thus therapeutic decisions are often delayed until substantial kidney function has been lost.

Progression to ESRD in patients with FSGS is caused by the constant loss of podocytes (specialized filtering cells of the kidneys). The loss of these cells results in the progression of scarring. Previous research indicates that podocyte loss can be monitored through urinary mRNAs (molecules that encode protein products). Urine podocyte mRNA measurement could be a useful clinical tool for both diagnostic and monitoring purposes; it's noninvasive and can be repeated over time to monitor disease progression and response to treatment. In two studies presented at the American Society of Nephrology conference, podocyte loss was monitored using two urine mRNAs (nephrin and podocin) and since kidney scarring is a major component of progression, urine TGFb1 mRNA, was also measured. TGF-beta is known to drive progressive scarring in kidney disease. Preliminary data from patients with glomerular diseases shows elevated levels of urine podocin,

nephrin and TGFb1. The amount reflects the severity of injury. While further studies are needed before this can used in the clinic, it provides some hope in the future treatment of patients with kidney disease[4,5].

Researchers in this field have made strides in different directions and the discoveries being made will allow for safer and earlier diagnosis of patients with kidney disease. Furthermore, these discoveries can allow physicians to more accurately evaluate the therapies used in the hopes that tailored treatments and medications can be applied to improve on patient care.

1. Omran Bakoush, Rafid Tofik, Rawa Aziz, Ahmed Reda, The Value of IgG-Uria in Predicting Renal Failure in Idiopathic Glomerular Diseases. A Long-Term Follow-Up Study. Presented at the American Society of Nephrology conference in San Diego, October 29 through November 1, 2009.

2. Jeroen Deegens, Ilse Rood, Micheal Merchant, Daniel Wilkey, Jack Wetzels, Jon Klein Identification of Biomarkers in Nephrotic Syndrome: Isolation of Urinary Exosomes. Presented at the American Society of Nephrology conference in San Diego, October 29 through November 1, 2009.

3. Michael R. Bennett, Michiko Suzuki, Prasad Devarajan, Kimberly Czech NGAL and MMP-7 as Differential Markers of Nephrotic Syndrome. Presented at the American Society of Nephrology conference in San Diego, October 29 through November 1, 2009.

4. Larysa T. Wickman, Sang Koo Lee, Akihiro Fukuda, Chrysta Leinczewski, Su Wang, Mahboob Chowdhury, Jocelyn Wiggins, David B. Kershaw, Matthias Kretzler, Roger C. Wiggins. Monitoring Progression of Kidney Disease through Urine mRNAs. Presented at the American Society of Nephrology conference in San Diego, October 29 through November 1, 2009.

5. Akihiro Fukuda, Yuji Sato, Madhusudan Venkatareddy, Larysa T. Wickman, Mahboob Chowdhury, Su Wang, Jocelyn E. Wiggins, Roger C. Wiggins, Urine TGF-beta1 mRNA and Podocyte Biomarkers for Progression. Presented at the American Society of Nephrology conference in San Diego, October 29 through November 1, 2009.

Quality of life in pediatric patients with FSGS

FSGS patients are often resistant to the available medical treatments and the prognosis for many of these patients is not promising, with a large percentage progressing to end stage kidney disease (ESKD). Given the circumstances, the physical and emotional toll that these patients face, it may not be surprising to learn that FSGS can significantly lessen ones quality of life. A recent study presented at the American Society of Nephrology conference attempted to provide more insight.

The FSGS Clinical Trial (FSGS-CT) was a large scale, multicenter trial comparing the efficacy of different medications in steroid resistant patients but also examined the quality of life of those enrolled. Of the 138 patients in the study, 64% were under the age of 18. Children between ages 8-18 (92%), completed a self report form regarding their quality of life while all parents completed a similar Parent form. Quality of life was divided into four areas, physical, emotional, social, and school functioning and the results of the completed surveys were compared to data in children with ESKD as well as healthy controls.

Review of the surveys found that quality of life in children with steroid resistant FSGS was significantly diminished. These children had lower scores for total quality of life in all of the above areas when compared to healthy children, except for social functioning. While the reduction in quality of life in physical functioning was comparable between children with FSGS and ESKD, emotional functioning was lowest in the FSGS group, even below children with ESKD.

Quality of life for FSGS patients is frequently overlooked but is a critical consideration when determining overall health. These findings indicate the need that medical care for children with steroid resistant FSGS include strategies to lessen the negative impact of the disease on their quality of life. Further study is necessary to determine if reduced quality of life is observed in other glomerular diseases and if it is due to clinical symptoms or side effects of medication used.

1. Howard Trachtman, R. Fine, A. Friedman, J. Gassman, D. Gipson, T. Greene, S. Hogan, R. Hogg, F. Kaskel, M. Moxey-Mims, M. Radeva, N. Siegel, S. Watkins. Quality of Life in Children with Focal Segmental Glomerulosclerosis (FSGS): Baseline Findings. Report of the FSGS-Clinical Trial (CT). Presented at the American Society of Nephrology, San Diego, October 29 through November 1, 2009.

Phase 1 Trial of Adalimumab for FSGS: Report of the FONT Study

Primary FSGS is one of the most common causes of non diabetic kidney disease and also one of the most difficult to treat due to the difficulty with initiating and maintaining remission. The standard of treatment has been the use of high doses of steroids given either daily or every other day. While these medications have been known to result in significant toxicity and are not without negative side effects, the lack of treatment predominantly progresses to end stage kidney disease (ESKD). Unfortunately, a number of patients with primary FSGS are resistant to current treatments and thus at high risk of progressing to ESKD.

While treatment options are limited for patients affected by FSGS, hope may be on the horizon with a category of drugs called antifibrotics. These medications attempt to decrease or prevent the development of fibrosis (scarring), which would help to preserve kidney function, decrease the level of proteinuria (leakage of protein into the urine) and delay or prevent progression to ESKD.

Recently, in the *American Journal of Kidney Diseases*, the results of a phase I clinical trial was published evaluating the safety and tolerability of adalimumab in patients diagnosed with steroid resistant FSGS. Adalimumab inhibits tumor necrosis factor (TNF), one of the major hormones produced by cells that induce inflammation. Inhibition of TNF is a possible pathway for treating FSGS, a promising strategy to slow or halt the decline in kidney function.

Ten patients diagnosed with steroid resistant FSGS were evaluated and given adalimumab every 14 days for 16 weeks, a total of 9 doses. Evaluation of glomerular filtration rate, proteinuria (leakage of protein into the urine), and pharmacokinetic assessment was completed. Adalimumab was found to be well tolerated with no serious adverse events or complications that could be attributed to the drug. Proteinuria decreased by at least 50% in 4 of the 10 treated patients.

There is a desperate need to find medications for the treatment of steroid resistant FSGS. This study provides a rationale to evaluate the efficacy of adalimumab as an antifibrotic agent for resistant FSGS in phase 2 and 3 clinical trials. The FONT II study will look at the efficacy of adalimumab in patients with steroid resistant FSGS. For more information or if you would like to enroll in the FONT II study please visit, http://clinicaltrials.gov/ct2/show/NCT00814255 or contact Dr.

Howard Trachtman, MD at 718-470-3423 trachtma@lij.edu or Dr. Debbie Gipson, MD at 919-966-2561 ext 235 debbie_gipson@unc.med.edu.

1. Joy MS, Gipson DS, Powell L, Machardy J, Jennette JC, Vento S, Pan C, Savin V, Eddy A, Fogo AB, Kopp JB, Cattran D, Trachtman H. Phase 1 Trial of Adalimumab in Focal Segmental Glomerulosclerosis (FSGS): II. Report of the FONT (Novel Therapies for Resistant FSGS) Study Group. AJKD 2010, January; 55(1): 55-60.

Management of childhood-onset nephrotic syndrome

The Study of Kidney Disease in Children (ISKD) in the 1970s was instrumental in the development of the approach taken in the treatment of children with primary nephrotic syndrome (NS). Unfortunately however, clinicians and researchers are finding a change in the characteristics of children diagnosed with NS, such as an increase in the number of children diagnosed with FSGS which is less responsive to steroid therapy when compared to minimal change disease (MCD). In addition to this, the increase in obesity and diabetes mellitus in children in the US may be adding to the difficulty of treating children with NS.

The Children's Nephrotic Syndrome Consensus Conference was formed to review how children with NS were being managed by their physicians and provide a guideline for treatment. When the literature was reviewed, it was found that the duration of initial steroid therapy ranged from 4 to 24 weeks and there was great variation in the therapies used when alternatives to steroids were considered[1].

While the researchers note that the variations in therapy witnessed here are not unusual in the care of children, they mention that a systematic approach to care and improvement in health outcomes can significantly improve outcomes in children with chronic conditions[1,2].

The final product was an educational guideline to aid pediatric nephrologists in the treatment of primary NS in children. Topics discussed include recommendations for the initial evaluation of patients and progression of therapy in these children to include: prednisone 2mg/kg per day for six weeks (with a maximum dose of 60mg), followed by prednisone 1.5mg/kg on alternate days for six weeks (with a maximum dose of 40mg) [2].

The authors provide information regarding therapy for patients that relapse following treatment as well as management of steroid resistant nephrotic syndrome. Additionally, management of complications such as obesity and growth, infection, thromboembolism and vaccination are clarified[2].

This major advance, an effort in standardizing care for all children affected by NS, will undoubtedly lead to improvements in care of these children and in time we will be able to measure the improvement in patient outcome.

2. MacHardy N, Miles PV, Massengill SF, Smoyer WE, Mahan JD, Greenbaum L, Massie S, Yao L, Nagaraj S, Lin JJ, Wigfall D, Trachtman H, Hu Y, Gipson DS. Management of childhood pattern onset of nephrotic syndrome. Pediatr Nephrol. 2009 Dec; 24(12):2393-400.

3. Gipson DS, Massengill SF, Yao L, Nagaraj S, Smoyer WE, Mahan JD, Wigfall D, Miles P, Powell L, Lin JJ, Trachtman H, Greenbaum LA. Management of childhood onset nephrotic syndrome. Pediatrics 2009, Aug; 124(2):747-757.

Characteristics of patients with FSGS, a look at the FSGS Clinical Trial

The FSGS Clinical Trial (FSGS-CT), a five year trial beginning in 2005, was the first large scale, multicenter, NIH funded trial, designed to compare the rate of remission of different medications in a population of patients diagnosed with steroid resistant FSGS. Additionally, the study has allowed a glimpse into the characteristics of those children and young adults involved.

The FSGS-CT enrolled 192 subjects of which138 were randomized in the trial from 66 participating sites. Based on review of the study participants, the patients enrolled were young and without other significant simultaneous health conditions. Of the patients enrolled, 30% were between the ages of 2 and 12 years, 34% were between the ages of 13 and 17 years, and 36% were 18 years or older. Furthermore, 38% of the patients enrolled were African American and 53% were male.

Perhaps not surprisingly, 51% of the patients involved had a history of hypertension and in fact, 20% of study participants failed to meet eligibility for the study at the initial screening based on blood pressure criteria. Their screening blood pressure values were greater than allowed by the eligibility criteria necessitating adjustments in medications and additional blood pressure monitoring.

Aside from hypertension, the majority of those enrolled reported experiencing symptoms, of which edema or swelling (57.2%), cough (26.8%), nausea (19.6%), diarrhea (18.8%) and vomiting (13%) were the most prevalent.

Among points of note were that 13% of the participants enrolled had a history of premature birth (defined as less than 37 weeks), and 15% had low birth weight. Other medical conditions analyzed included attention deficit disorder (5.8%) and a history of seizures (5.1%). When asked about smoking history, the researchers found that 7.2% were current smokers, 20.3% were passive smokers and 6.5% were former smokers. While impact of smoke exposure is unknown at this time, it may be an important factor in the patients' response to treatment.

Analysis of the trial is expected to be completed shortly at which point researchers will be better able to define the impact of patient characteristics, disease history and smoke exposure on the patient response to treatment.

1. Debbie S. Gipson, M. Radeva, R. Fine, A. Friedman, J. Gassman, T. Greene, R. Hogg, F. Kaskel, M. Moxey-Mims, N. Siegel, H. Trachtman, S. Watkins, Focal Segmental Glomerulosclerosis Clinical Trial (FSGS-CT) Study Cohort. Presented at the American Society of Nephrology Conference, October 29 through November 2, 2009.

Is vitamin D a factor in kidney disease?

Vitamin D deficiency is a very common problem in the United States, particularly in the winter months when sunlight is often harder to come by. Studies have linked vitamin D deficiency to a variety of different problems ranging from diabetes and high blood pressure to cancer, heart disease and even mood disorders. Researchers from Albert Einstein College of Medicine have now provided a link between low levels of vitamin D and end stage kidney disease (ESKD).

It has been known for some time that there is a high prevalence of vitamin D deficiency in those with chronic kidney disease and for this reason vitamin D is often administered to these patients. While the mechanism of action remains unknown, administration of vitamin D has been associated with a survival benefit[1].

In a study by Dr. Melamed and colleagues, the link between vitamin D deficiency and race was evaluated. It is well known that African Americans have a disproportionate burden of both FSGS and ESKD when compared to the Caucasian population. Following analysis of more than 13,000 people, including measurements of vitamin D, the researchers found that participants with the lowest levels of vitamin were more likely to end up on dialysis. Overall, 34% of African Americans had vitamin D levels below 15 nanograms per milliliter (ng/ml) compared to 5% of the non-Hispanic Caucasian population. Furthermore, those with a vitamin D level less than 15ng/ml had a 2.6 fold greater incidence of ESKD[2].

While the study suggests that vitamin D deficiency may be a key factor in the development of ESKD in African Americans, the researchers are not able to prove a cause and effect relationship. Additional studies are required to validate these findings and more importantly determine how vitamin D helps in the preservation of kidney function.

1. Cheng S, Coyne D, Vitamin D and outcomes in chronic kidney disease. Curr Opin Nephrol Hypertens. 2007 Mar; 16(2):77-82.

2. Michal L. Melamed, Brad Astor, Erin D. Michos, Thomas H. Hostetter, Neil R. Powe, Paul Muntner, Hydroxyvitamin D Levels, Race, and the Progression of Kidney Disease. Presented at the American Society of Nephrology conference in San Diego, October 29 through November 1, 2009.

The other kind of steroid: Anabolic steroid abuse leads to FSGS

It is well known that anabolic steroid use is not good for your health and in fact has been documented to result in the disruption of the normal hormones produced in the body, in a wide variety of symptoms including breast development in men, reduced fertility, behavioral changes and more significantly, cardiovascular and liver disease. Now however a link between the use of anabolic steroids and FSGS has been elucidated.

A study by researchers from Columbia University Medical Center identified a group of 10 bodybuilders who developed FSGS following long term use of anabolic steroids. These patients presented clinically with severe proteinuria (leakage of protein into the urine) and reduced kidney function. Nine of the patients underwent a kidney biopsy, and were found to have more aggressive forms of FSGS than is typically seen with secondary FSGS.

Eight patients were followed for an average of 2.2 years during which time one had progressed to end stage kidney disease (ESKD). Of these eight patients, seven discontinued the use of anabolic steroids and found a stabilization or improvement of their creatinine (an indicator of kidney function) and a reduction in their proteinuria. Perhaps the most interesting aspect of the study however being that one patient restarted anabolic steroid use, against the recommendations of his physicians, and experienced a relapse of proteinuria and reduced kidney function.

The researchers do however provide other explanations that may be involved in the development of FSGS. While the use of anabolic steroids is believed to have a direct toxic effect on these patients, the bodybuilders were also typically consuming a mix of growth hormone, insulin, diuretics and protein shakes all of which could have added to the effect of the anabolic steroids. Furthermore, the researchers propose that the increase in muscle mass in these patients put additional stress on their kidneys, requiring the kidneys increase the rate of filtration and effectively harming them.

1. Leal C. Herlitz, Glen S. Markowitz, Alton B. Farris, Joshua A. Schwimmer, Michael B. Stokes, Cheryl Kunis, Robert B. Colvin, Vivette D. D'Agati. Development of Focal Segmental Glomerulosclerosis after Anabolic Steroid Abuse**.** Presented at the American Society of Nephrology conference, San Diego, October 29 through November 1, 2009.

A link between soft drinks and kidney disease

Researchers at Brigham and Women's Hospital in Boston have recently presented a study indicating the positive correlation between the effects of sugar and artificially sweetened beverages on a decline in kidney function. While previous research has indicated an association between consumption of sugar sweetened beverages with an increase in diabetes there was no previous data on the effect of these beverages on kidney function.

The researchers identified over 3,000 women who had data on sugar sweetened beverages or artificially sweetened soda intake and glomerular filtration rate (an indication of kidney function) between 1989 and 2000. The average age of the women in the study was 67 years and 97% were Caucasian. Fifty-four percent of the women had hypertension and 24% were diabetic. When different variables such as age, hypertension, body mass index, diabetes, cigarette smoking, physical activity, cardiovascular disease and caloric intake, were adjusted for, the researchers discovered that the consumption of two or more servings per day of artificially sweetened soda was associated with a 30% or greater decline of glomerular filtration rate over 11 years. In effect the consumption of two or more servings per day of artificially sweetened soda was associated with a two-fold increase in the risk for kidney function decline[1].

1. Julie Lin, Gary C. Curhan, Associations of Sweetened Beverages with Kidney Function Decline. Presented at the American Society of Nephrology conference in San Diego, October 29 through November 1, 2009.

TRPC6 mutations and Nephrotic Syndrome

In 2005, our knowledge of the genetics associated with FSGS expanded with the publication of an article in the journal *Science* with the discovery of a mutation in the gene TRPC6, encoding a calcium ion channel. Mutations in the channel, located on the cell membrane of podocytes (specialized cells in the kidneys involved in filtration), were found to result in an aggressive form of FSGS in adults. This was an interesting discovery by Dr. Michelle Winn particularly because it provided an alternative explanation for development of FSGS than was previously known[1]. Since its discovery a great deal of research has focused on elucidating the role of mutations in TRPC6 and FSGS.

Until recently, seven different mutations in TRPC6 were identified as a cause of FSGS in adults. In a study from Heeringa and colleagues yet another mutation in the TRPC6 gene was found that leads to early onset FSGS, affecting children as early as nine years of age. This data came after the group analyzed 550 families with steroid resistant nephrotic syndrome worldwide. The researchers found that the mutation discovered led to an aggressive form of FSGS in these children[2].

The mutation in the channel is called a gain of function mutation because it causes an increase in calcium into the cell but the mechanism behind the mutation and the development of FSGS are still a mystery. Possible explanations provided are that the mutant TRPC6 may affect interactions with the podocyte proteins leading to abnormalities or they may amplify injuries present. In a study by researchers in Boston, the interactions of TRPC6 have been evaluated, and they have identified SNF8 as a regulator of TRPC6, suggesting that modulating this interaction can influence the ability of the TRPC6 mutations to cause FSGS[4].

To further determine the role of TRPC6 channels, researchers have begun to examine its function and how that is affected when the gene is mutated. Calcium influx into podocytes has been described previously in response to signaling by other compounds (Angiotensin II), but the channels responsible for the influx of calcium were unknown. A study by Dr. Jacobo and colleagues determined that TRPC6 appears to be the elusive calcium channel in the podocyte and thus it likely plays a role in podocyte injury. This observation suggests that maintenance of calcium balance may be critical, since loss of channel activity may have deleterious effects on the specialized cells of the kidney[6].

While researchers do not yet know exactly how these mutations result in disease, there are a number of talented scientists trying to elucidate the role of TRCP6 in FSGS. As research continues there is hope that these mutations can be a potential target of therapy for FSGS patients to ameliorate the disease.

1. Winn MP, Conlon PJ, Lynn KL, et al. A mutation in the TRPC6 cation channel causes familial focal segmental glomerulosclerosis. *Science* 2005;

2. Heeringa SF, Möller CC, Du J, Yue L, Hinkes B, Chernin G, Vlangos CN, Hoyer PF, Reiser J, Hildebrandt F. A novel TRPC6 mutation that causes childhood FSGS. PLoS One. 2009 Nov 10; 4(11):e7771.

3. N Mukerji, TV Damodaran, MP Winn, TRPC6 and FSGS: The latest TRP channelopathy. *Kidney International* **76**, 1225–1238 (1 December 2009)

4. Johannes S. Schlondorff, Robert Carrasquillo, Anna Greka, Martin R. Pollak **SNF8, a** Component of the ESCRT-II Complex, Binds and Regulates Wild-Type and FSGS-Associated Mutant TRPC6. Presented at the American Society of Nephrology conference in San Diego, October 29 through November 1, 2009.

5. Jason J. Eckel, Nirvan Mukerji, Peter Lavin, Naila Ferimazova, Rasheed Gbadegesin, Tirupapuliyur Damodaran, Brandy Bowling, Guanghong Wu, Alison Homstad, Laura Barisoni, Bartlomiej Bartkowiak, Michelle P. Winn Medicine, TRPC6 Deficiency Does Not Cause Glomerulosclerosis. Presented at the American Society of Nephrology conference in San Diego, October 29 through November 1, 2009

6. Sarah M. P. Jacobo, David Billing, Wen Chih Chiang, Arnolt Ramos, Dequan Tian, Jochen Reiser, Hsiang-Hao Hsu, Hermann Pavenstaedt, Anna Greka, TRPC6 Channel Signaling in Response to Angiotensin II Type 1 Receptors Is Essential for the Preservation of the Podocyte Cytoskeleton. Presented at the American Society of Nephrology conference in San Diego, October 29 through November 1, 2009.

7. Rasheed A. Gbadegesin, T. V. Damodaran, Alison Homstad, Bartlomej Bartkowiak, Brandy Bowling, Guanhong Wu, Peter Lavin, Jason Eckel, Nirvan Mukerji, Michelle Winn, TRPC6 Gene Deficiency Ameliorates the Course of Puromycin Induced Kidney Injury. Presented at the American Society of Nephrology conference in San Diego, October 29 through November 1, 2009.
8. Tom Nijenhuis, Joost Hoenderop, Jan Flesche, Harry van Goor, Marinka Bakker, Rene Bindels, Gerjan Navis, Jack Wetzels, Jo Berden, Jochen Reiser, Johan van der Vlag, Angiotensin II-Mediated Upregulation of TRPC6 Expression Via Calcineurin/NFAT Signalling in Podocyte Injury. Presented at the American Society of Nephrology conference in San Diego, October 29 through November 1, 2009.

Transplantation in patients with FSGS, long term outcomes and advances

While great strides have been made in our understanding of the biological mechanisms underlying FSGS, the therapies used have not changed substantially. The mainstay of therapy continues to be long term use of harsh steroids and without response to therapy the majority of patients can expect progression to kidney failure[1]. While a transplant of the diseased kidney gives hope when dealing with resistant FSGS, an estimated 30% of patients experience recurrence of the disease in their transplanted kidney. Little information is available regarding the long term results of kidney transplantation in adults with FSGS[2].

In an attempt to provide greater information regarding long term effects, Moroni and colleagues followed 52 patients after transplantation. The transplants were performed between 1988 and 2008 and were compared to 104 matched controls. After 15 years, all patients had survived but when comparing graft survival, the researchers found that only 57% of the grafts in the FSGS patients had survived compared to 88% of grafts in the control population. FSGS recurred in 12 of 52 grafts and led to graft failure within an average of 10 months in seven of the patients. In the remaining five patients, proteinuria (leakage of protein into the urine) remitted and the grafts continued to function. Furthermore, following re-transplantation in 8 patients, five experienced a second recurrence. When reviewing the data, the researchers found that patients who experienced recurrence were more frequently male, younger at diagnosis of FSGS and younger at transplant[3].

Recurrence of FSGS following transplantation is a problem that researchers are trying to alleviate through various medications and therapies. In a study by Dr. Straatmann and colleagues the efficacy of early initiation of prolonged plasmaphoresis (PP) in pediatric transplant patients with recurrent FSGS was examined. Following a review of 21 patients who underwent a kidney transplant for primary FSGS, the researchers found that 5 patients (24%) experienced recurrence in the first week following surgery. All of these patients received immunosuppressive medications and two additionally received cyclophosphamide. All five patients started PP between 2 and 11 days after transplantation which was discontinued when the patients achieved remission, ranging between 4 to 24 weeks. Two of the five patients had a second recurrence of FSGS but in both, recurrence remitted with additional PP (ranging from 3-7 weeks). The researchers

report that PP was 100% successful in achieving remission in patients with a recurrence of FSGS which they attribute to early diagnosis of the recurrence following transplantation, early initiation of PP and prolonged treatment with PP[6].

While Dr. Straatman has had success with the use of PP in patients with recurrence there are cases in which neither immunosuppressive medications nor PP can achieve remission. In a study published in the *American Journal of Transplantation,* the case of a 15-year-old boy with FSGS leading to end-stage kidney disease (ESKD) was presented. The patient presented with an early post-transplant recurrence of FSGS and following immunosuppressive treatment and PP the researchers noted only partial and transient control of his FSGS. In an attempt to improve on the patients' condition, the researchers began anti-TNF-alpha treatment (infliximab then etanercep). TNF-alpha is involved in the inflammatory process and its inhibition is believed to be a possible pathway for treatment of FSGS to slow or halt kidney function decline. Following this treatment, the researchers noted a significant decrease in proteinuria but once treatment was discontinued the patient experienced a relapse of disease[4].

Given the number of difficult cases that present following transplantation, the question was raised whether a preemptive kidney transplant would be beneficial for patients with FSGS. In a study presented at the American Society of Nephrology conference in San Diego, outcome measures including patient death, graft loss, acute rejection within six months of transplant, and delayed graft function (DGF) rates, were compared between preemptive and non-preemptive transplant recipients diagnosed with FSGS. The study analyzed 11,024 patients who underwent kidney transplant between 1987 and 2007. Of these patients, 1,942 patients were transplanted preemptively. In combined analysis, the preemptive group had a lower adjusted risk of death, graft loss and DGF compared to the non-preemptive group. The study provides some insight into the possible benefit of preemptive kidney transplantation in patients with FSGS[5].

The studies presented here are a testament to the need for greater research into the mechanisms underlying the FSGS disease process. It is with greater knowledge of the disease that we can hope for improved treatment options.

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Mutations in INF2: A new discovery in FSGS genetics

Research into FSGS and nephrotic syndrome has come a long way in the last decade and as 2009 comes to a close, another major discovery excites both the nephrology community as well as all of the families affected by these diseases. Discovery of a new mutation in a gene previously unknown to cause FSGS promises to provide greater insight into FSGS, increasing our understanding of this once enigmatic disease.

The discovery of multiple mutations in the gene INF2 and its correlation with FSGS was made by Dr. Elizabeth Brown of Children's Hospital Boston in association with Dr. Martin Pollak of Brigham and Women's Hospital. The discovery was made using a genetic linkage analysis, a procedure in which a comparison is made between family members affected with a disease and those that are unaffected. Comparison of two large families with multiple members affected by FSGS led the researchers to mutations in the gene INF2 and after sequencing 91 additional unrelated families it was found that there were mutations in INF2 in 11 of these families as well.

The gene in question, INF2, is found within the podocytes, specialized kidney cells involved in filtration. A great deal of research indicates that podocytes are essential for filtration and disruption of the structure of the cells results in disease such as the case with FSGS. INF2 is known to encode a protein that regulates actin. Actin, a protein, is also found within podocytes and is important in maintaining the architecture of these cells. Dr. Brown and colleagues suggest that mutations in INF2 within podocytes results in a disruption in their structure and in effect a disruption in filtration by the kidneys.

As research continues into INF2 we hope to learn more about the biology behind FSGS and what causes it. Through this knowledge and understanding comes the possibility of improving the treatment options for those affected by FSGS.

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European Collaborative FSGS Transplantation Study (ECoFTS)

Collaboration is defined as coming together to work towards a common vision. Within science and medicine this involves the dedication to discovery and commitment to solving many of the key challenges faced by researchers and physicians and the patients that they treat. With the increasing number of scientific discoveries and the vast amount of knowledge that accumulates in any given year, collaboration among researchers and physicians is necessary to discern the discoveries that hold potential for future applications in patient care. FSGS is one such disease that requires collaboration by the scientific community. Given the low prevalence of FSGS, it is imperative for physicians and researchers to come together to try to better understand this disease.

In an attempt to further our understanding of FSGS, the European Collaborative FSGS Transplantation Study (ECoFTS), a multicenter study, has brought together researchers and physicians from hospitals in Austria, Germany, Canada, the Czech Republic, France, Norway, Pakistan, Poland, Switzerland and the United States. ECoFTS has been established to evaluate the growing problem of FSGS internationally, targeting children with primary relapsing FSGS, particularly to evaluate therapies following transplantation in these patients. This international collaboration is aimed at finding whether there is a causal correlation between mutations in previously discovered genes and disease progression and relapse of the underlying disease following transplantation as well as to evaluate which therapies can improve prognosis of FSGS before and after transplantation.

Through the collection of data from doctors treating FSGS internationally, as well as collection of blood and urine specimens from patients, the researchers will try to answer the above questions. The samples collected will be tested for mutations in two genes, NPHS2 and TRPC6 and the urine samples will be used to find specific proteins that could be used as biomarkers for FSGS. The study could allow the improved diagnosis of FSGS as well as provide information as to how to best treat this disease.

Questions regarding the study or if you would like to learn how to get involved please contact:

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Rituximab, a clinical update

Since the discovery that the drug rituximab could hold potential as a rescue therapy for patients affected by steroid resistant and dependent forms of FSGS and nephrotic syndrome (NS) there has been growing interest to learn more about it by both the scientific and lay communities.

Researchers have long suspected that T cells, a type of white blood cell important in immune response, plays a role in the development of NS [1] and now, there is increasing evidence that B cells, another category of white blood cells, may play an important role in NS as well[2].

Rituximab is an antibody directed against an antigen (receptor) found on B cells. When administered, it results in reduction of the number of B cells. Treatment with rituximab has been successful in B cell lymphomas, resulting when B cells are mutated and become cancerous, as well as in patients with autoimmune diseases[3].

In an effort to further examine the efficacy of rituximab on patients diagnosed with idiopathic NS, Dr. Prytula and colleagues collected data from questionnaires provided by members of the International Society of Paediatric Nephrology. The researchers received 70 questionnaires and divided the data into three groups. Group 1 was comprised of steroid dependent and frequently relapsing patients, group 2 included those diagnosed with steroid resistant NS and group 3 were children treated with rituximab for recurrence following transplantation. Within these categories, researchers found that 82% of the patients in group 1 experienced a good initial response with 61% experiencing a complete remission. Group 2 was less positive with 44% of these patients having an initially positive response and 22% achieving complete remission. Finally, in the patients diagnosed with post-transplant recurrence of NS, treatment with rituximab resulted in an initial positive response in 60% while 40% achieved full remission[4].

A separate study evaluated the efficacy of rituximab in five patients with FSGS with variable results. The objective of the study by Dr. Printza and colleagues was to assess the efficacy of rituximab in five children diagnosed with steroid resistant FSGS all of whom had failed conventional therapies and did not have a genetic form of the disease. Rituximab was provided at a dose of 375mg/m2 and the patients were followed up weekly to evaluate B cell depletion. Patients who did

not have an initial decrease in their B cell count or who failed to achieve remission were given up to four additional doses of rituximab. The researchers found that 2 of these five patients achieved and maintained a complete remission of FSGS. Another two patients experienced partial remission, however one was not responsive at follow up. The final patient presented did not respond to rituximab treatment[5]. These results are further indication of the variability in treatment with rituximab but also indicate the potential for its use in some patients with difficult to treat FSGS.

Another study of note was presented at the European Society of Paedtric Nephrology conference in the United Kingdom this year. Dr. Strologo and colleagues evaluated the use of rituximab in patients diagnosed with FSGS following a relapse after kidney transplantation. The authors presented the effects of rituximab on seven children who experienced a relapse of disease following transplantation and who had failed to respond to intensive plasmapheresis. The team found that leakage of protein was halted in three patients receiving treatment with rituximab, while another patient experienced a 70% decrease in protein leakage and yet another had a 50% decrease. Of the remaining patients, one did not respond to treatment and reached kidney failure within three months and the final patient had a severe reaction a few minutes following treatment with rituximab[6].

To date, information is available from only a small number of patients and the positive results may be overestimated due to publication bias. Overall, the review of the literature suggests that while rituximab may provide physicians with another option as a rescue treatment for patients with NS, larger, controlled trials are needed to determine the true value and efficacy of rituximab. More information is needed in order to determine which patients are likely to benefit and what the risks of treatment may be.

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Evaluating the use of corticosteroids and cyclosporine in treatment of NS

A diagnosis of nephrotic syndrome (NS) is a difficult one for many families to cope with and understandably results in a number of questions regarding available treatments as well as both short and long term outcomes. These questions are often asked of researchers and clinicians and while we do not yet have all of the answers there is a growing body of research evaluating the effects of available treatments.

The mainstay of therapy for NS continues to be the use of corticosteroids and medications that suppress the immune system. While the use of these medications results in remission of disease in a large number of patients, it is well known that these medications can be difficult to tolerate and they are not without side effects.

A study by Feber and colleagues evaluated the use of corticosteroids on skeletal changes in children diagnosed with NS. While steroids are known to have harmful effects on bone development and health, the authors sought to learn more about the skeletal effects on children with NS following short term use of steroids, within 30 days of initiation of treatment and three months following initiation. Sixty-three children diagnosed with primary NS were evaluated and the authors found that bone mineral density was reduced in these children as little as thirty days into treatment and as treatment continued over three months, bone mineral density continued to decline. Furthermore, three of the patients were found to have developed mild vertebral fractures within the first 30 days of treatment. This study suggest that even short term use of steroids on children with NS may have adverse effects on bone health[1].

Cylcosporine A (CsA) is another well established medication used in the treatment of NS. CsA was introduced as a treatment for idiopathic NS over 20 years ago, particularly effective for those patients with steroid-responsive NS who suffer from multiple relapses as well as for patients with steroid resistant forms of the disease. CsA is an immunosuppressive drug used in the treatment of these conditions because of its ability to reduce proteinuria, the leakage of protein into the urine[2]. CsA may be effective in the treatment of NS but at the same time there are well-documented side effects and the potential nephrotoxicity found with long term use. A study by Wafo and colleagues evaluated the prevalence of nephrotoxicity in children with steroid dependent NS given CsA from 1990 to 2008. The

researchers found that of 71 kidney biopsies that were evaluated (after an average of more than three years of treatment with CsA), 22 experienced nephrotoxicity, equating to 31%. The majority of the cases (17/21) were mild and none were severe. Additionally, the data suggest treatment of hypertension with ACE or ARBs while simultaneously using CsA put the patients at higher risk for nephrotoxicity[3].

While the negative effects of many of the available treatments for NS and FSGS can at times make us question their use, the prognosis may be much worse if these diseases are left untreated. More than half of untreated patients with FSGS will progress to end stage kidney disease (ESKD)[4]. These studies are a testament to the need for continued evaluation of commonly used therapies and of course for the continued effort in development of new treatments.

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