The role of animal models in the understanding and treatment of focal segmental glomerulosclerosis: a review of the literature

S.M.L. de Mik, M.J. Hoogduijn, R.W.F. de Bruin, F.J.M.F. Dor

Sylvana ML de Mik¹
Email: sdemik@gmail.com

Martin J Hoogduijn, PhD³
Email: m.hoogduijn@erasmusmc.nl

Ron W de Bruin, PhD¹
Email: r.w.f.debruin@erasmusmc.nl

Frank JMF Dor, MD PhD¹,²
Email: f.dor@erasmusmc.nl

¹ Laboratory of experimental surgery, Department of Surgery, Erasmus MC, University Medical Center, Rotterdam, The Netherlands

² Department of surgery, Erasmus MC, University Medical Center, Rotterdam, The Netherlands
Abstract:

Background
Focal segmental glomerulosclerosis (FSGS) is a kidney disease with progressive glomerular scarring and a clinical presentation of nephrotic syndrome. FSGS is with 4% the most common primary glomerular disorder that causes end-stage renal disease. 40% of patients have recurrence of FSGS after kidney transplantation. 80% of FSGS is idiopathic and the disease develops over time.

Results
In this review, a variety of animal models used to study FSGS are discussed. These animal models include the following: remnant kidney model developed by resecting 5/6 of renal mass; reduction of renal mass due to systemic disease for example hypertension, hyperlipidemia or SLE; drug-induced FSGS using adriamycin, puromycin or streptozotocin; virus-induced FSGS most commonly HIV-1; and genetically-induced FSGS such as Mpv-17, α-actinin 4 and Thy-1.1. In addition an animal model for spontaneous developing FSGS is discussed.

Conclusion
To date, there is no exact understanding of the pathogenesis of idiopathic FSGS, and there is no definite curative treatment. One requirement facilitating FSGS research is an animal model that resembles human FSGS. Most animal models used to study FSGS induce secondary forms of FSGS in an acute manner. The ideal animal model for primary FSGS should mimic the human primary form in that it has a chronic development of FSGS, which is slow and develops spontaneously. Such models are, however, rare. We conclude that is a need for a better animal model to investigate pathogenesis and potential treatment options of FSGS.

**Keywords**

Focal segmental glomerulosclerosis, animal model, remnant kidney, adriamycin, puromycin aminonucleoside-induced nephrosis, hiv, Mpv-17, α-actinin 4.

**Background**

Focal segmental glomerulosclerosis (FSGS) is a disease with progressive glomerular scarring. Studies[1, 2] have shown that mainly podocytes are involved in the development of FSGS. Podocytes are epithelial cells of the visceral layer of the kidney’s Bowman’s capsule. Their function is to form a filtration structure preventing protein loss. When destruction of podocytes occurs, by any form of cellular stress, it results into sclerosis of part (segmental) of the glomerular capillaries in a minority (focal) of glomeruli. If
the sclerosis continues, global glomerulosclerosis will develop. Clinically, this loss of podocytes and their filtration function results in nephrotic syndrome, consisting of proteinuria, hypoalbuminemia, hypercholesterolemia and peripheral edema.[3]

FSGS has an incidence of 7 per million, and is in 20% of children and 40% of adults, respectively, the underlying cause of nephrotic syndrome. When presented with high proteinuria levels, 50% of cases progress to end-stage renal disease (ESRD) within 3 to 8 years, making FSGS causal for 4% of all ESRD cases.[3] After KTx (KT), the FSGS recurrence rate is 40% [4]

In 80% of FSGS patients, the disease is primary (idiopathic). In the remaining 20%, many secondary forms of FSGS have been discovered. Genetic causes (α-actinine 4 mutations) were found, as well as forms that were virus-associated (HIV), drug-induced (interferon-α), caused by reduced renal mass (unilateral agenesis) or by reduction of renal mass due to systemic disease (hypertension).[3]

Ever since FSGS was first described by Arnold Rich in 1959[5], many studies have been conducted to understand the pathogenesis of FSGS and to identify risk factors and/or possible treatments for this disease. In order to facilitate the study of FSGS, different kinds of animal models have been developed to mimic the clinical pathological features of FSGS. In this review, an overview will be given of different animal models that are currently being used to investigate FSGS, or have been used in the past. The vast majority of animal
models induce FSGS by employing a variety of approaches, varying from reducing renal mass to the use of drugs and viruses, and the introduction of various genetic mutations.

**Animal models for FSGS**

*Remnant kidney model*

The most frequently used animal model is the reduced or remnant kidney model. In these rodent models of mouse or rat origin, 5/6 of renal mass is resected. To compensate for the loss of renal mass, tubular and glomerular growth occurs. Glomerular growth is achieved by both hyperplasia and hypertrophy. Podocyte growth is structurally slower, as it occurs only through hypertrophy. Therefore, both the capillary and filtration area for a single podocyte is dramatically enlarged. As a consequence, the filtrate cannot be filtered into the urinary space fast enough, causing a blockage diverting the filtrate into the space between the podocyte body and foot processes. These maladaptive changes eventually lead to cell destruction and adhesions between the glomerular basement membrane (GBM) and Bowman's capsule causing sclerosis.[6]

Studies using this animal model are conducted for the development of preventive treatment strategies as well as for gaining more insight in underlying pathologies. Using the remnant kidney model it was found that inhibition of tromboxane synthesis[7], the administration of clofibric acid (lipid-lowering agent)[8], troglitazone (peroxisome proliferator-activated receptor-gamma agonist)[9] and Tranilast (antifibrotic agent)[10] can all ameliorate
progressive glomerulosclerosis. Other studies show that absence of functional p21(WAF1/CIP1) genes can reduce progression to chronic renal failure[11] and that apolipoprotein E knockout mice do not have an increase in renal injury after subtotal nephrectomy in the presence of hyperlipidemia,[12] suggesting a role in the development of secondary FSGS for this protein.

The remnant kidney model is often used in combination with other induced FSGS models, such as injections with puromycin or with induced hypertension, FSGS models to be discussed in the next paragraphs.

Renal mass reduction due to systemic disease
The reduction of renal mass is a secondary event to certain pathologies. In a number of animal models, the decrease in renal mass is the result of chronic damage to the glomerular vessels due to hypertension. In these models, FSGS develops in a similar fashion as in the remnant kidney model, where a decrease in renal mass is observed leading to less glomeruli to filtrate the same high amount of serum to be filtrated. Hypertension in these models can be observed in salt-sensitive animals[13] or renal hypertension can be caused by administration of norepinephrine or angiotensin II.[14] In addition, hyperlipidemia and obesity models have been investigated (Zuckerrats)[15] as well as ageing models (Munich Wistar rats).[16] Besides observing the effect of hypertension on the development of glomerulosclerosis in these two animal models, the Zuckerrats show that early influx of glomerular macrophages precedes glomerulosclerosis.[15] The aging Munich Wistar rats show that age-dependent glomerulosclerosis is reversed after endothelin-1 inhibition. Endothelin-1 seems to have a negative effect on podocytes cell-cycle activity
and dedifferentiation. When administering an endothelin-1 antagonist podocytes may re-enter the cell-cycle and recover from previous and age-related injury.[17]

More acute damage to glomerular vessels can occur due to antiphospholipid antibodies from systemic lupus erythematosus (SLE)[18] or sickle-shaped red blood cells during a sickle cell crisis that occlude the glomerular vessels and result in inflammation.[19] TNF-α is found to protect the kidney in SLE mice.[18]

Drug-induced

Adriamycin, puromycin, streptozotocin are the drugs mostly used to induce FSGS. Additionally, the literature describes a small number of studies conducted with cyclosporine[20] and growth hormone[21], which will not be discussed here.

Adriamycin is known as an oncolytic antibiotic that can induce proteinuria from the second infusion onward, when given intravenously at 2mg/kg in a 3-week interval. After 16 weeks, segmental glomerulosclerosis is observed with progression to global glomerulosclerosis and tubulointerstitial fibrosis at 24 weeks. Due to increased serum urea levels, some of the animals will not survive to 28 weeks. When given in a single intravenous dose of 5 mg/kg, adriamycin causes sclerosis within 6 months in 50% of animals.[22]

Puromycin is an antibiotic that inhibits protein synthesis. Puromycin can be given by multiple intraperitoneal injections with initial injection of 10 mg/kg
followed by 40 mg/kg every 4 weeks or as a single intravenous dose of 50 mg/kg to cause puromycin aminonucleoside-induced nephrosis (PAN). After injection, rats show an early nephrotic phase peaking at 10 days with complete foot process-effacement followed by apparent resolution. Between 10 and 13 weeks, progressive lower-level proteinuria develops with early segmental sclerotic lesions leading to well-defined segmental sclerosis at 18 weeks.[22]

The direct effect of both drugs is that they exert oxidative damage directly to the podocytes, either by reduction of a semiquinone radical, or by producing reactive oxygen species [13]. These ROS interfere with α-3 integrin expression, a key molecule for maintaining podocytes’ shape and adhesion. Alteration of α-3 integrin may cause foot process effacement and alteration of cell-cell and cell-matrix interactions in these epithelial cell injury animal models.

Puromycin also causes overproduction of H₂O₂ and downregulation of the podocyte proteins podoplanin and nephrin, which are important elements of foot processes and slit diaphragms.

Both adriamycin and puromycin are used frequently to induce FSGS because of strong dose-response effects.[22] These drugs are often used in the same study in two separate arms. The models have been used to study serial micropuncture analysis of a single nephron while glomerulosclerosis is developing. [23] FSGS treatment studies for which adriamycin and puromycin animal models are used show that the combination of ACE-inhibitors and
angiotensin I blockers do not have a better effect than ACE-inhibitors alone.[24] In addition, it shows that MAPK is essential for podocyte injury making p38 MAPK a potential therapeutic target[25] and that vaccination with CCL2 DNA protects against kidney injury after adriamycin injections.[26] Possible new biomarkers for initiation and severity of FSGS, such as fibronectin[27] and Rab 23[28] respectively, were studied in these animal models as well. Serum fibronectin levels can show a slight but significant increase 3 days before the occurrence of glomerular fibronectin deposits making it a non-specific biomarker for predisposition of FSGS.[27] In case of Rab 23, an autocrine signalling pathway is observed in mesangial cells, while developing FSGS, leading to elevated urine levels of Rab 23 suppressing this pathway. Therefore, as a biomarker, Rab 23 urine levels could indicate the severity of FSGS.[28]

Streptozotocin is a naturally occurring chemical, which is toxic to insulin-producing beta-cells of the pancreas. It can be used to treat cancers of the Islets of Langerhans[29] and in medical research to induce diabetes in animal models.[30] Intraperitoneal injection (40mg/kg) of hamsters gives an ongoing hyperglycaemia and hyperlipidaemia with high glucose urine levels, resulting in glomerular lipidosis after 1 month. After 3 months, FSGS with mesangial expansion is seen. This is caused by an increase of basement membrane-like material, lipid droplets and foam cells. Especially, the hyperlipidaemia is crucial in this development since it forms the lipid droplets.[31] Studies using streptozotocin-induced hyperglycaemia in rodents show that doxazin, a blood pressure lowering agent, reduces albuminuria with 80%, but
does not have an effect on mesangial expansion or progression to glomerulosclerosis unlike good glycemic control which prevents all three.[28] Altered gene expression in the early phase of kidney disease caused by hyperglycaemia may be critical in these animals.[32]

**Virus-induced**

Virus induced animal models that are most often used in FSGS research are HIV-1 based models, in which transgenic mice express HIV-1 accessory genes such as Viral protein R (Vpr). Vpr mediates epithelial growth phase 2 cell-cycle arrest and apoptosis in tubular cells of the kidney.[33] In addition, macaques infected with cloned lymphocyte tropic simian immunodeficiency virus are used to study FSGS.[34] The virus can inflict damage on podocytes, either by direct infection of these cells or by the release of inflammatory cytokines. The virus can furthermore transfer from infected T-cells to tubular epithelial cells via viral synapses during cell adhesion. Damage to the kidney can persist, despite antiretroviral therapy. Studies using this animal model have demonstrated protection and reversal of glomerulosclerosis in mice treated with Fluvastatin[35] and cyclin-dependent kinase inhibitor CYC202[36], respectively.

**Genetic targets of FSGS**

Recent articles[1, 2] identified podocytes as the major cellular target in developing FSGS, resulting in new approaches for developing animal models. Proteins that compose the podocyte and slith diaphragm were discovered. Genes encoding for these proteins were then targeted to develop knock-out
mouse models for FSGS. Mpv-17 and α-actinin 4 mutations were used most frequently. Models such as podocin-defecient mice were only described once in literature.[37]

Mpv-17 inactivation by retroviral insertion gives foot process flattening and proteinuria within 30 days postpartum caused by an excessive production of radical oxygen species and accumulation of lipid peroxidation adducts. After 9-12 months the mice die from kidney failure.[38] Studies using Mpv-17 inactivation show mitochondrial DNA depletion leading to skin, inner ear and kidney involvement. At the onset of FSGS hardly any mitochondrial DNA is left in the cells of the glomerular tuft.[39]

The α-actinin 4-gene encodes for the production of an actin cross-linking protein. Point mutations in this gene cause an autosomal dominant form of human FSGS. There is significant reduction of mRNA and nephrin, a component of the slit diaphragm. The result is a rapidly degrading and deregulating actin cytoskeleton caused by α-actinin-4 and deterioration of the slit diaphragm caused by nephrin, leading to early development of proteinuria and FSGS.[40] In studies with α-actinin 4 mutated mice samples are used for comparison with the autosomal dominant form of human FSGS caused by the same α-actinin 4 mutation.[40]

A different way to use a transgenic mouse model is by using mice that express the Thy-1.1 antigen on podocytes. Thy-1.1 is not expressed on podocytes in normal mice. After injecting Anti-Thy-1.1 monoclonal antibodies,
podocytes are damaged and acute albuminuria is induced within a day, accompanied by a rapidly developing focal glomerulosclerosis at day 21.[41] Studies using the Thy-1.1 transgenic mouse demonstrate it is a good model to specifically study the relation between podocyte injury, albuminuria and FSGS development, since it has been proven that in this model the severity of FGSG is related to the amount of podocyte injury. [41] The animal model is also used to study the participation of STAT3 and Smad1 activation in development of FSGS[42] and to study the effect of combined treatment with rosuvastatin and an angiotensin I blocker.[43]

Spontaneous developing FSGS

In the literature, only one idiopathic mouse model that spontaneously developed FSGS has been published. Articles of this FGS/Nga mouse model have appeared between 1991 and 2004. The mouse model was established after interstrain crossbreeding of CBA/Nga and RFM/Nga offspring. The strain spontaneously developed FSGS lesions at 3 months and severe glomerulosclerosis within one year. Studies of this mouse model revealed dense deposits in the mesangium containing IgA, IgM, C3 and the retroviral envelope antigen. Breeding of these animals was possible up to 18 generations. [44]

A study using this mouse model showed that bone marrow transplantation (BMT) from normal mice to FGS mice ameliorates FSGS and that BMT or transfer of purified hemopoietic stem cells from FSG mice to normal mice induced FSGS.[45] A different study was also conducted to locate quantative trait loci (QTL) affecting the glomerulosclerosis index (GSI) in these mice. Two
QTL were found on chromosomes 8 and 10. The presence of Gsi1 increased GSI and the presence of Gsi2 decreased GSI.[46]

Currently, there are only some embryos left of this mouse model in a biolaboratory in Japan, but no active research seems to be performed. [44]

<table>
<thead>
<tr>
<th>Animal Models</th>
<th>Method of developing</th>
<th>Examples of FSGS research conducted using these animal models</th>
</tr>
</thead>
</table>

Table 1: overview of animal models discussed in this review

Conclusion
A lot of our knowledge on FSGS has come from a variety of animal models. However, to date, there is still no exact understanding of the pathogenesis of idiopathic FSGS, and there is no definite curative treatment even with KT. Therefore much research on FSGS still needs to be done. Especially due to the donor shortage, the frequent need for re-transplantation in FSGS patients, as well as the side effects of immunosuppression, improvements are required for treatment of this disease. Our inability to exactly understand the pathogenesis and find curative treatment for FSGS may be due to the fact that almost all of the animal models used are based on the induction of secondary forms of FSGS (described above) and most of them give an acute onset of proteinuria and FSGS, whereas in human FSGS 80% is idiopathic which develops as a chronic disease over time. To facilitate better research, one requirement is an animal model that resembles human FSGS. The ideal animal model for primary FSGS should mimic the human primary form of FSGS, which has a spontaneous onset and develops chronically. Such models, as described in the previous paragraph, are, however, rare.

**List of abbreviations**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>FSGS</td>
<td>Focal segmental glomerulosclerosis</td>
</tr>
<tr>
<td>ESRD</td>
<td>End-stage renal disease</td>
</tr>
<tr>
<td>KT</td>
<td>Kidney transplantation</td>
</tr>
<tr>
<td>HIV</td>
<td>Human immunodeficiency virus</td>
</tr>
<tr>
<td>GBM</td>
<td>Glomerular basement membrane</td>
</tr>
<tr>
<td>SLE</td>
<td>Systemic lupus erythematosus</td>
</tr>
</tbody>
</table>
TNF- α: Tumor necrosis factor-alfa
PAN: Puromycin aminonucleoside-induced nephrosis
ROS: Reactive oxygen species
H$_2$O$_2$: Hydrogen peroxide
ACE-i: Angiotensin-converting-enzyme inhibitor
MAPK: Mitogen-activated protein kinases
CCL2: Chemokine (C-C motif) ligand 2
DNA: Deoxyribonucleic acid
Vpr: Viral protein R
CD4: Cluster of differentiation 4 T-helper cells
mRNA: Messenger ribonucleic acid
STAT3: Signal transducer and activator of transcription 3
IgA: Immunoglobulin A
IgM: Immunoglobulin M
C3: Complement component 3

**Competing interests:**

Regarding this review on used animal models for FSGS, we declare to have no financial disclosures.

**Authors’ contributions**

SM drafted the manuscript. FD, MH and RB revised and approved the final manuscript.
References:


