

CYCLODEXTRIN PROTECTS PODOCYTES IN FOCAL SEGMENTAL GLOMERULOSCLEROSIS (FSGS)

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BACKGROUND

Focal segmental glomerulosclerosis (FSGS) is the most common primary glomerular disease leading to end-stage kidney disease (ESKD) due to glomerulonephritis in the US, particularly in children and young adults. Approximately 2000 individuals reach ESKD each year. The estimated life-time risk for FSGS is 0.17% in European Americans and 0.72% in African Americans [1]. Susceptibility to FSGS in African Americans is associated with genetic variants of the APOL1 gene (G1 and G2) [2].

It has become clear in the last few years that FSGS represents a heterogeneous group of diseases. The Nephrotic Syndrome Study Network (NEPTUNE) study has created a novel individualized approach to profile proteinuric patients using morphological and expression profiles descriptors. The NEPTUNE study has also allowed us to identify key molecular pathways that are dysregulated in clinical FSGS, thus allowing for a more clinically meaningful effort in designing related experimental studies.

The pathogenesis of FSGS is associated with podocyte injury ultimately leading to glomerulosclerosis and end-stage kidney disease [3-7]. Podocytes contribute to the glomerular filtration barrier through a tight regulation of actin cytoskeleton remodeling [8,9]. However, the mechanisms leading to podocyte injury in FSGS remain poorly understood. Glomeruli from patients with type 2 diabetes and diabetic kidney disease (DKD), where podocyte injury is an early finding and podocyte loss (podocytopenia) is an independent predictor of disease progression [5], demonstrated a modulation of several genes primarily involved in cholesterol efflux. While cholesterol may play an important role in granting proper localization of slit diaphragm proteins, accumulation of cellular cholesterol may negatively affect podocyte function [10].

In support of a causative role of cellular cholesterol in the pathogenesis of podocyte injury, we have recently demonstrated that sequestration of cellular cholesterol with hydroxypropyl-beta cyclodextrin (CD) may protect from proteinuria in experimental DKD [10]. Whether cellular cholesterol may affect podocyte function in diseases other than DKD remains to be established. With this preliminary study, we have therefore tested the hypothesis that lipid related genes are modulated in FSGS and that CD treatment protects from experimental FSGS.

HYPOTHESIS

Lipid related genes are affected in glomeruli of patients with primary focal segmental glomerulosclerosis (FSGS) and treatment with cyclodextrin protects podocytes in experimental FSGS.

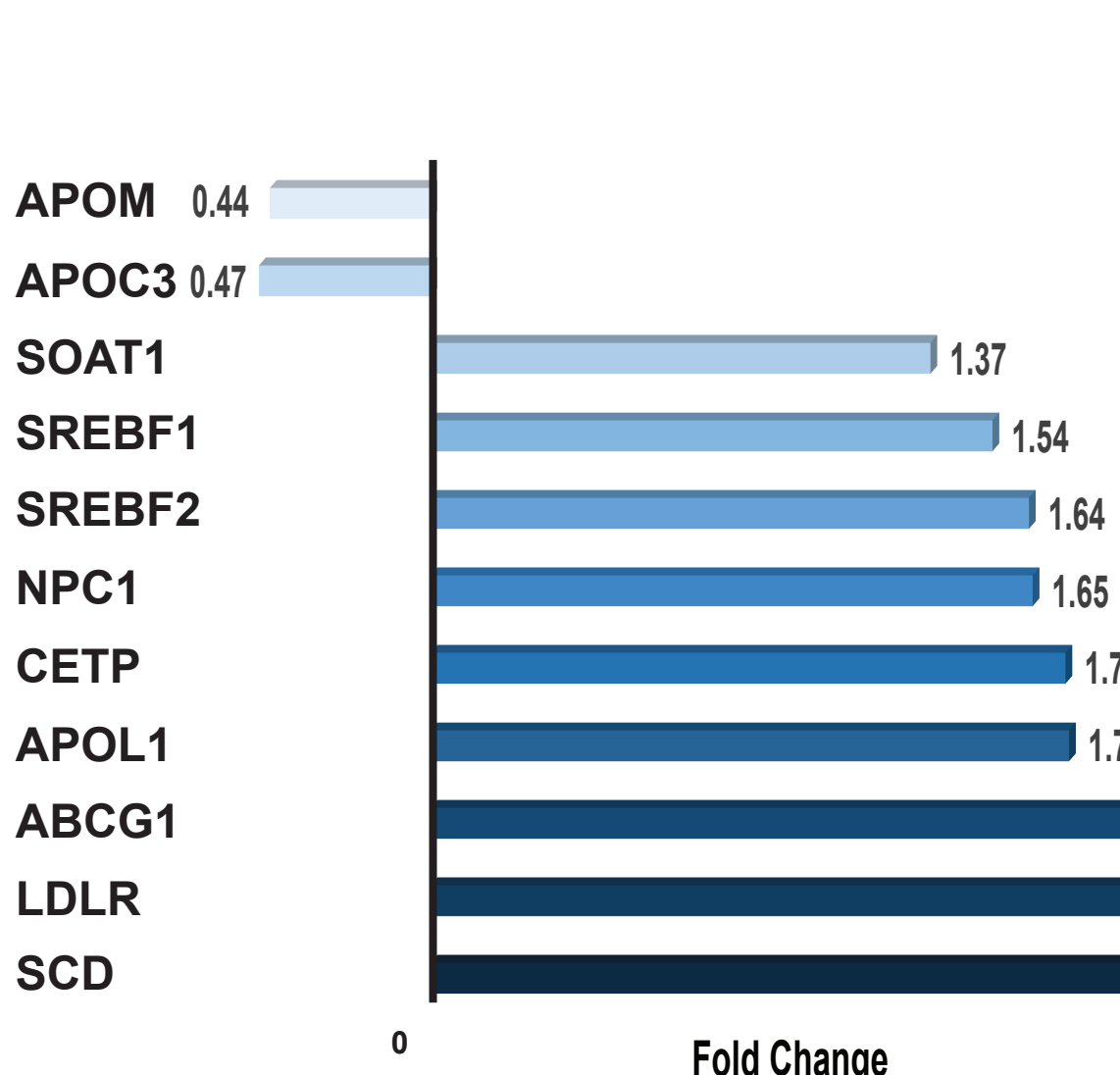
METHODS

Patients and data recruitment. Kidney biopsies were obtained from 54 patients with FSGS and 6 normal living donors from the NEPTUNE study, which is longitudinal observational cohort looking at individuals with nephrotic syndrome. **Microarray analysis.** Glomerular mRNA expression profiles of 84 related genes were studied in 54 patients with FSGS. The biopsy tissue specimens were manually microdissected as previously described [11, 12]. Glomerular gene expression profiling was performed using Human GenomeST2.1 Affymetrix Gene Chip arrays and processed using Affymetrix Power Tools. Differential expression analysis between the controls and relevant disease group was performed using Significance Analysis of Microarray as implemented in the TIGR Multiexperiment Viewer software suite. **The animal model of FSGS.** 5-week-old BALB/c female mice were purchased from the Jackson Laboratory. Mice were injected with a single intravenous dosage of adriamycin (11 mg/kg) to develop FSGS-like lesions. 24 hours after adriamycin injection, osmotic pumps (Alzet, 28 days) with 2-hydroxy-propyl-β-cyclodextrin (CD) in 0.9% saline solution (40 mg/kg/day) were implanted under the skin for 10 weeks. Four experimental groups (5 animals per group) were analyzed: control, ADR only (ADR), CD only (CD), and ADR plus CD (ADR+CD). Measurements of body weight and urine collections for ACR (albumin/creatinine ratios) determinations were performed weekly. At time of sacrifice, serum creatinine and Blood Urea Nitrogen (BUN) were determined by mass spectrometry and ELISA respectively, and perfused kidneys were collected for histological analysis. All animal procedures were conducted under protocols approved by the Institutional Animal Care and Use Committee. **Histology analysis.** Periodic acid-Schiff (PAS) or hematoxylin and eosin (H/E) staining of 4 micron-thick tissue sections were performed according to the standard protocols. Mesangial expansion area was determined as area of PAS staining. **Urinary albumin and creatinine** were determined as described by using mouse specific ELISA (Bethyl Lab.) and creatinine kits. **Serology.** Blood samples collected from mice at sacrifice were analyzed for creatinine level by mass spectrometry at the UCSD O'Brien Core Center (University of Alabama). Blood urine nitrogen (BUN) levels were measured by ELISA in the Comparative Laboratory Core facility of the University of Miami. **Statistical analysis** was implemented using GraphPad Prism Software (version 5). Analysis of variance (ANOVA) followed by Bonferroni's post-test or Student t-test was used.

RESULTS

Many Lipid Related Genes are Altered in Glomeruli Affected by FSGS

A Cholesterol Efflux Related Genes



B Lipid Metabolism Related Genes

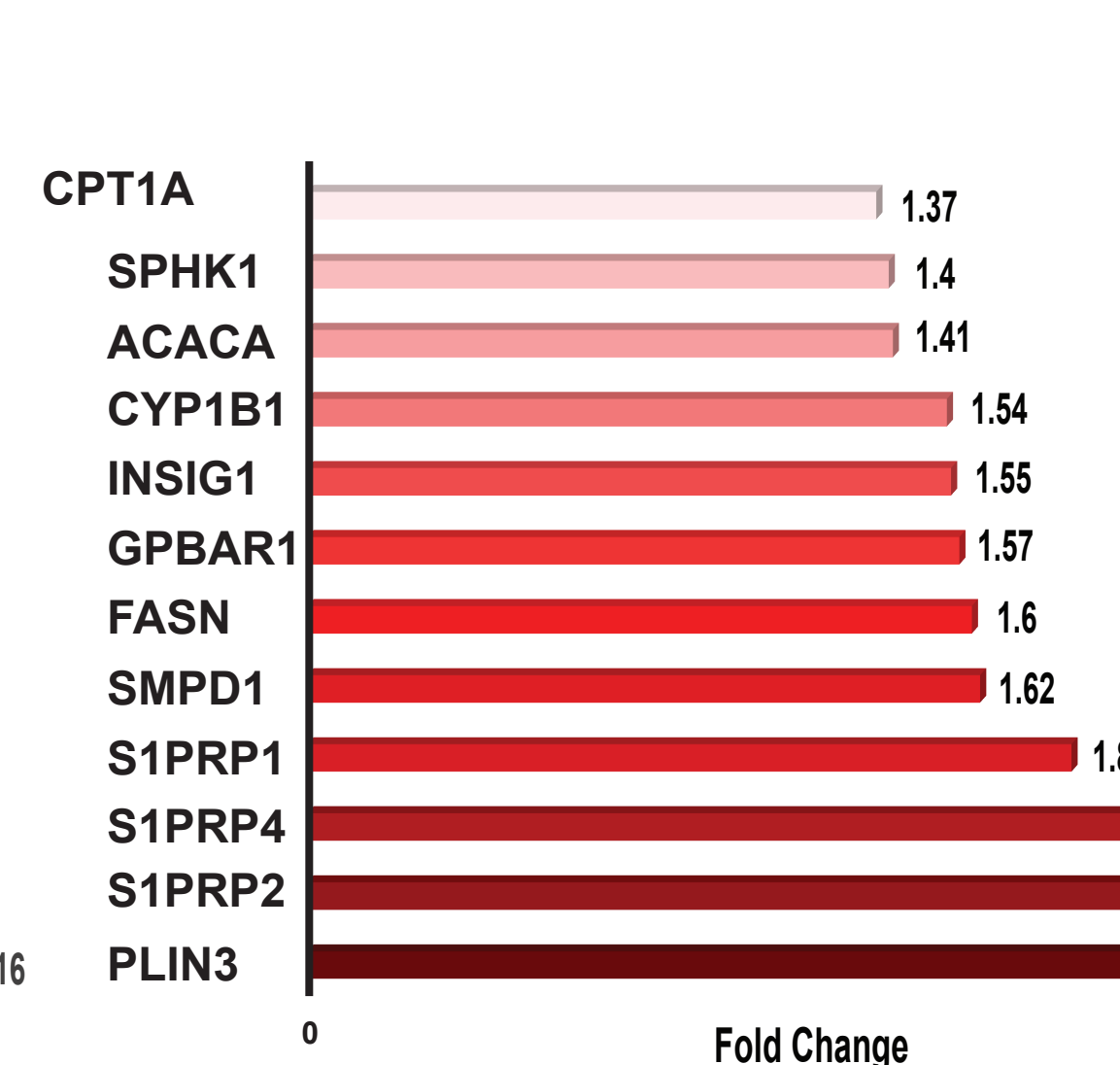


Figure 1. Microarray analysis of the glomerular transcripts in patients with FSGS enrolled in the NEPTUNE cohort. (A) We observed increased expression of many cholesterol efflux related genes (specific), however expression of apolipoprotein C-III (APOC3) and apolipoprotein M (APOM) genes were decreased. SCD - stearoyl-CoA desaturase, LDLR - low density lipoprotein receptor, ABCG1 - ATP-binding cassette, sub-family G, member 1, APOL1 - apolipoprotein L1, CETP - cholesteryl ester transfer protein, NPC1 - Niemann-Pick disease, type C1, SREBF1 or SREBF2 - sterol regulatory element binding transcription factor 1 or 2, SOAT1 - sterol O-acyltransferase 1. (B) Expression of many lipid dysmetabolism related genes (general) was increased. PLIN3 - perilipin, S1PR1, S1PR2 or S1PR4 - sphingosine-1-phosphate receptor 1, 2 or 4, SMPD1 - sphingomyelin phosphodiesterase 1, acid lysosomal, FASN - fatty acid synthase, GPBAR1 - G protein-coupled bile acid receptor 1, INSIG1 - insulin induced gene 1, CYP1B1 - cytochrome P450, family 1, subfamily B, polypeptide 1, ACACA - acetyl-CoA carboxylase alpha, SPHK1 - sphingosine kinase 1, CPT1A - carnitine palmitoyltransferase 1A (liver).

Expression of Lipid Related Genes Correlated with ApoL1

2 APOL1 risk alleles

Name	Symbol	P-value	Association
Cholesteryl Ester Transfer Protein, Plasma	CETP	0.01	Direct
ATP-binding cassette, sub-family A (ABCA1), member 1	ABCA1	0.01	
ATP-binding cassette, sub-family G (WHITE), member 8	ABCG8	0.01	
Fatty acid synthase	FASN	0.02	
Niemann-Pick disease, type C2	NPC2	0.04	
Apolipoprotein E	APOE	0.04	Inverse

0 APOL1 risk alleles

Name	Symbol	P-value	Association
Nuclear receptor subfamily 1, group H, member 2	NR1H2	0.02	Direct
Perilipin 2	PLIN2	0	Inverse
Fatty acid binding protein 1, liver	FABP1	0	
Lipase, endothelial	LIPG	0.01	
Apolipoprotein E	APOE	0.01	
Sterol O-acyltransferase 1	SOAT1	0.02	
Acyl-CoA Oxidase 2, Branched Chain	ACO2	0.02	Inverse
Apolipoprotein C-III	APOC3	0.03	
Sphingomyelin phosphodiesterase, acid-like 3A	SMPDL3A	0.03	
Perilipin 5	PLIN5	0.04	

Cholesterol efflux related genes (specific) Lipid dysmetabolism related genes (general)

Figure 2. Lipid dysmetabolism in relation to APOL1 expression. A different set of lipid related transcripts correlated with ApoL1 expression in patients carrying two risk alleles when compared to patients carrying no risk alleles. ApoL1 expression in glomeruli from patients with two risk alleles had primarily a positive correlation with lipid related genes, while ApoL1 expression in glomeruli from patients with no risk alleles had primarily a negative correlation with significant lipid related genes.

Cyclodextrin Protects Podocytes in Experimental FSGS *in vivo*

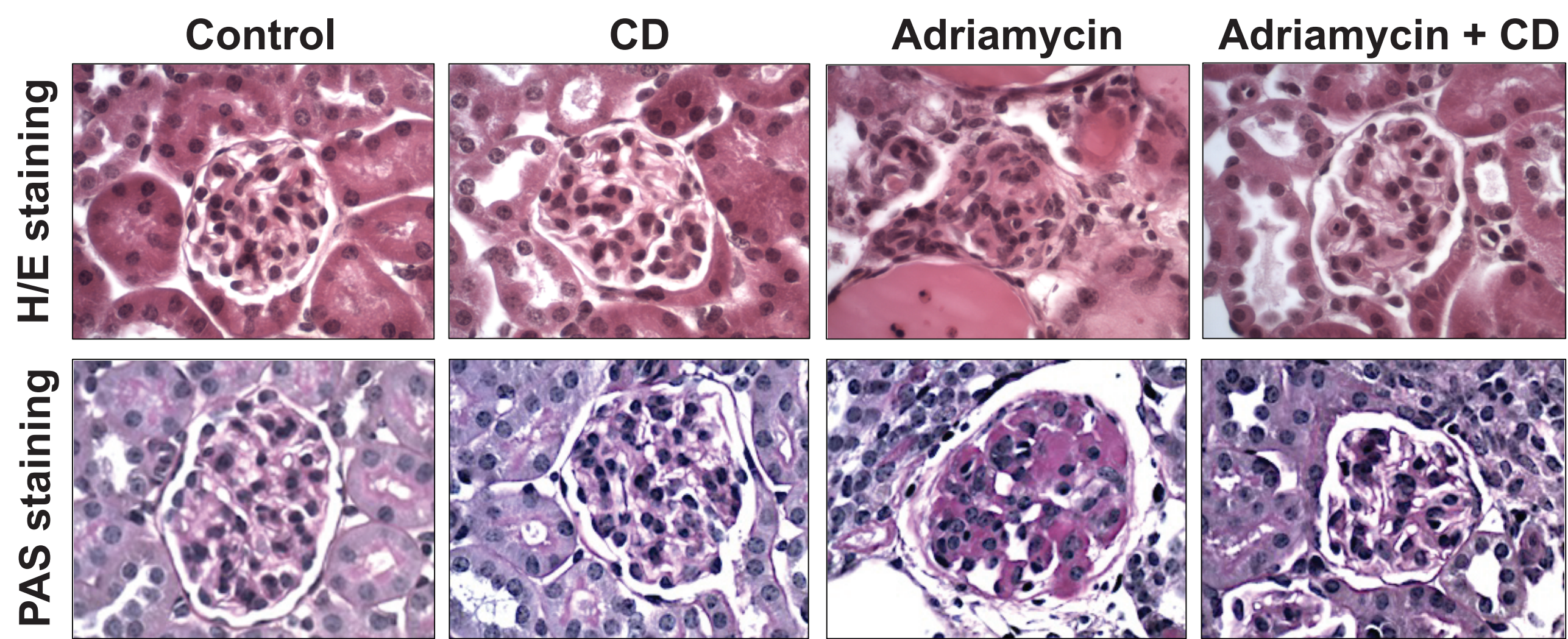


Figure 3. Histological analysis (H/E and PAS) of glomeruli in experimental model of FSGS *in vivo*. Four experimental groups of 5-week-old BALB/c female mice were analyzed: control (n=5), 2-hydroxypropyl-beta-cyclodextrin (CD, 40 mg/kg per day) only (n=5), adriamycin only (n=5) in dosage 11 mg/kg, and adriamycin+CD (n=5). Adriamycin treated mice are characterized by significantly increased mesangial expansion, which is completely prevented after CD administration.

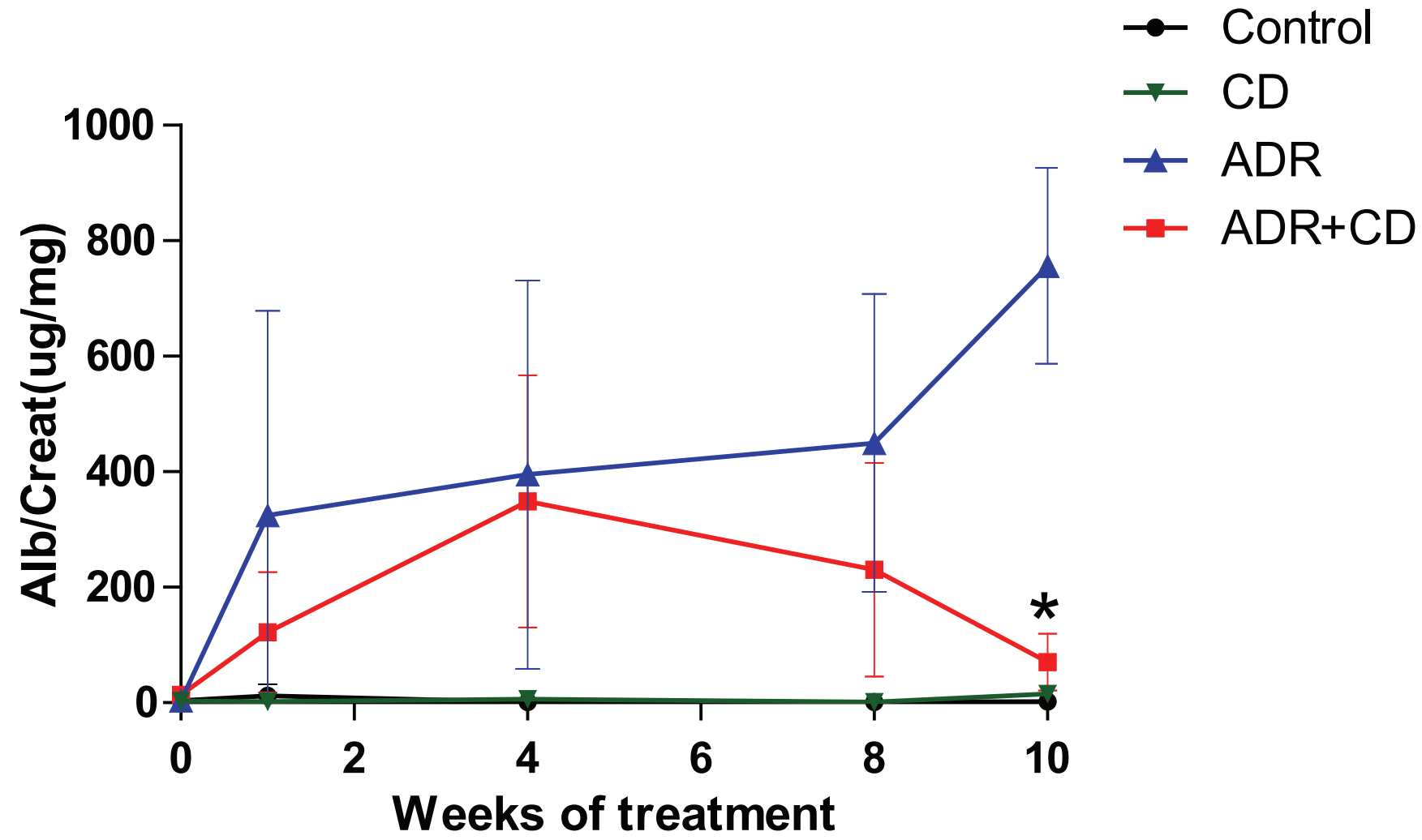


Figure 4. Adriamycin (ADR) administration causes albuminuria *in vivo* and cyclodextrin (CD) treatment protects from the development of albuminuria. Acute adriamycin administration of BALB/c female mice intravenously results in high albuminuria over one week. Treatment with cyclodextrin prevents development of albuminuria in mice in 10 weeks. *p<0.05 (t-test) when comparing adriamycin + cyclodextrin (ADR+CD) group of mice to adriamycin (ADR) group of mice.

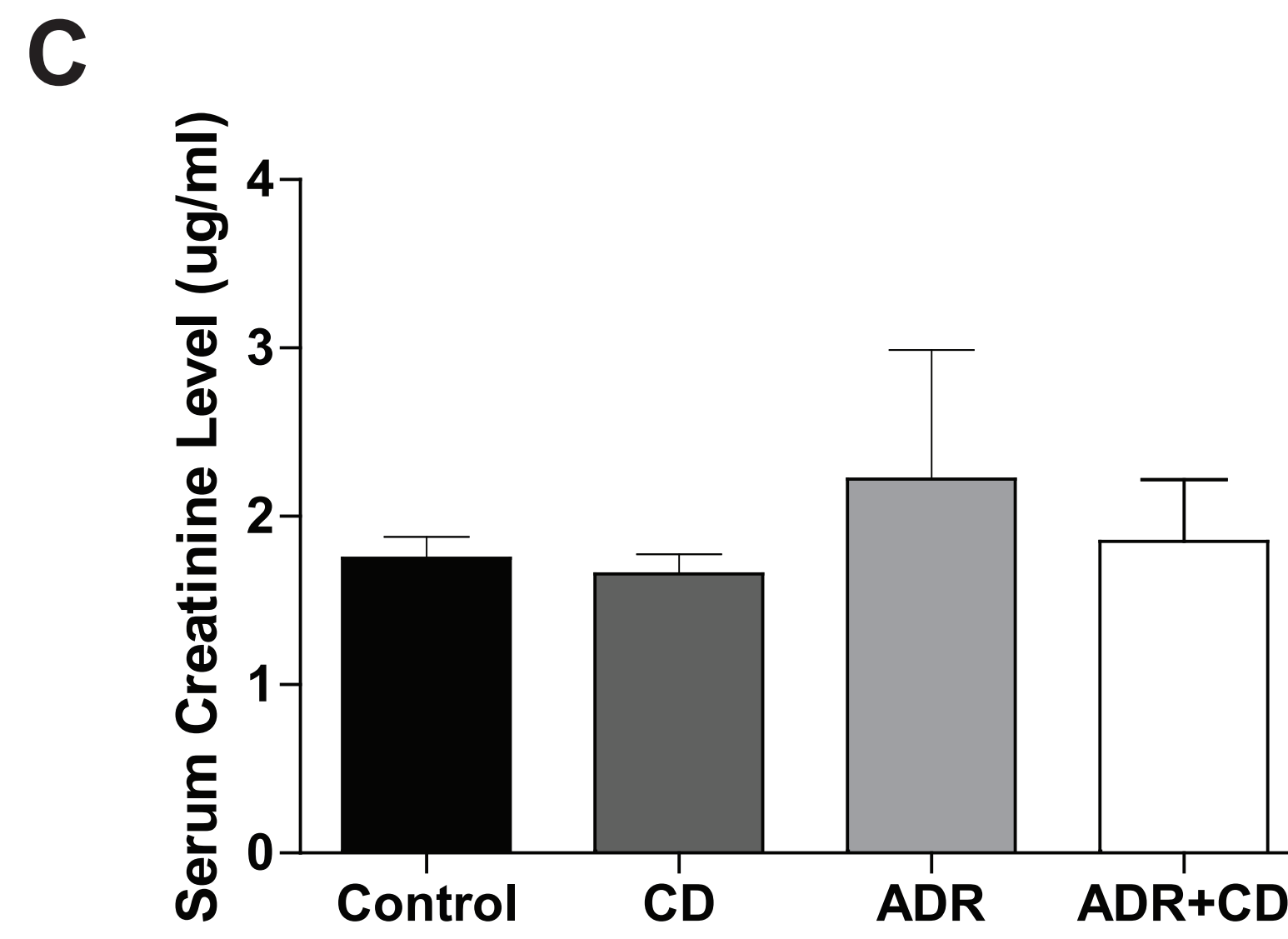
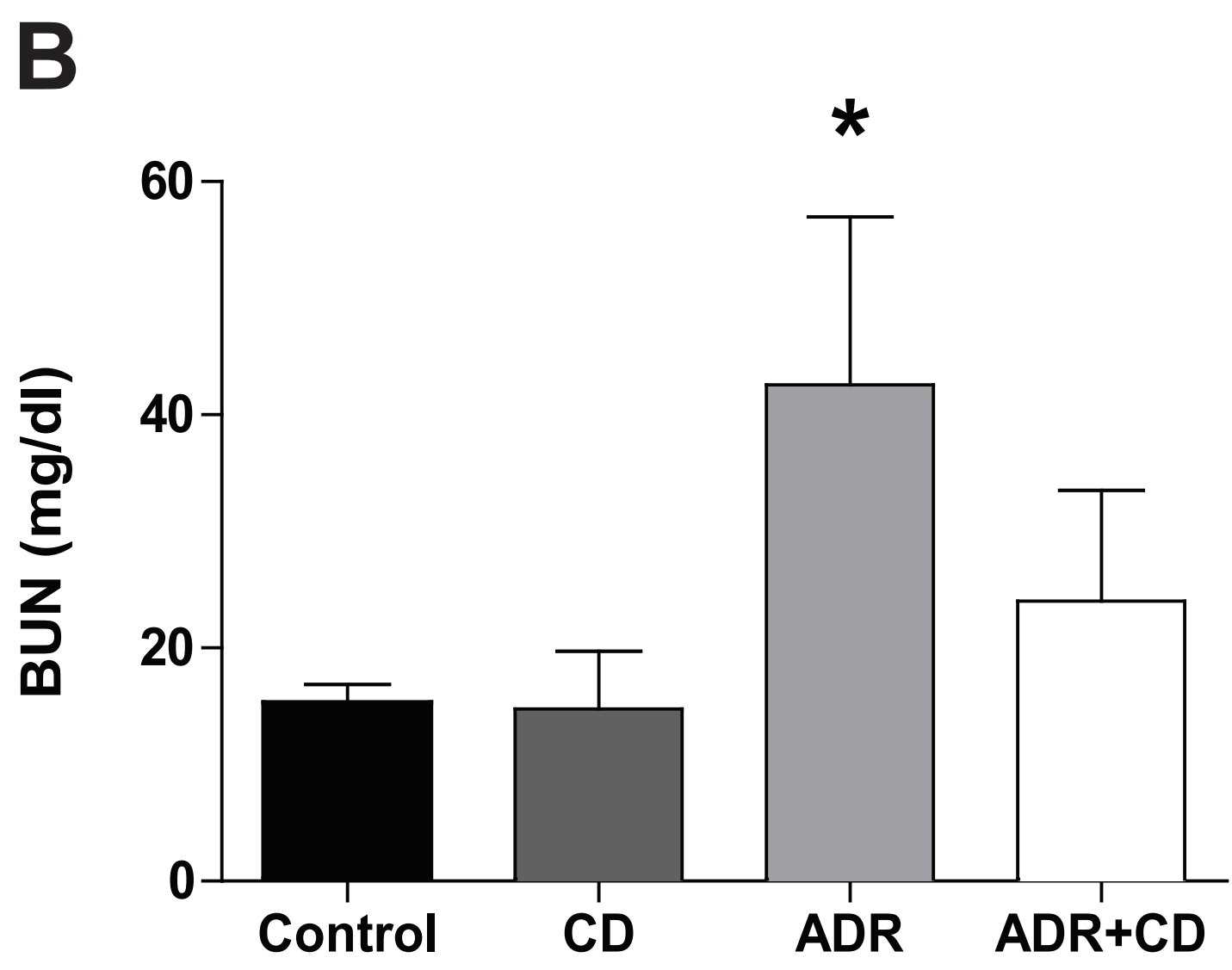
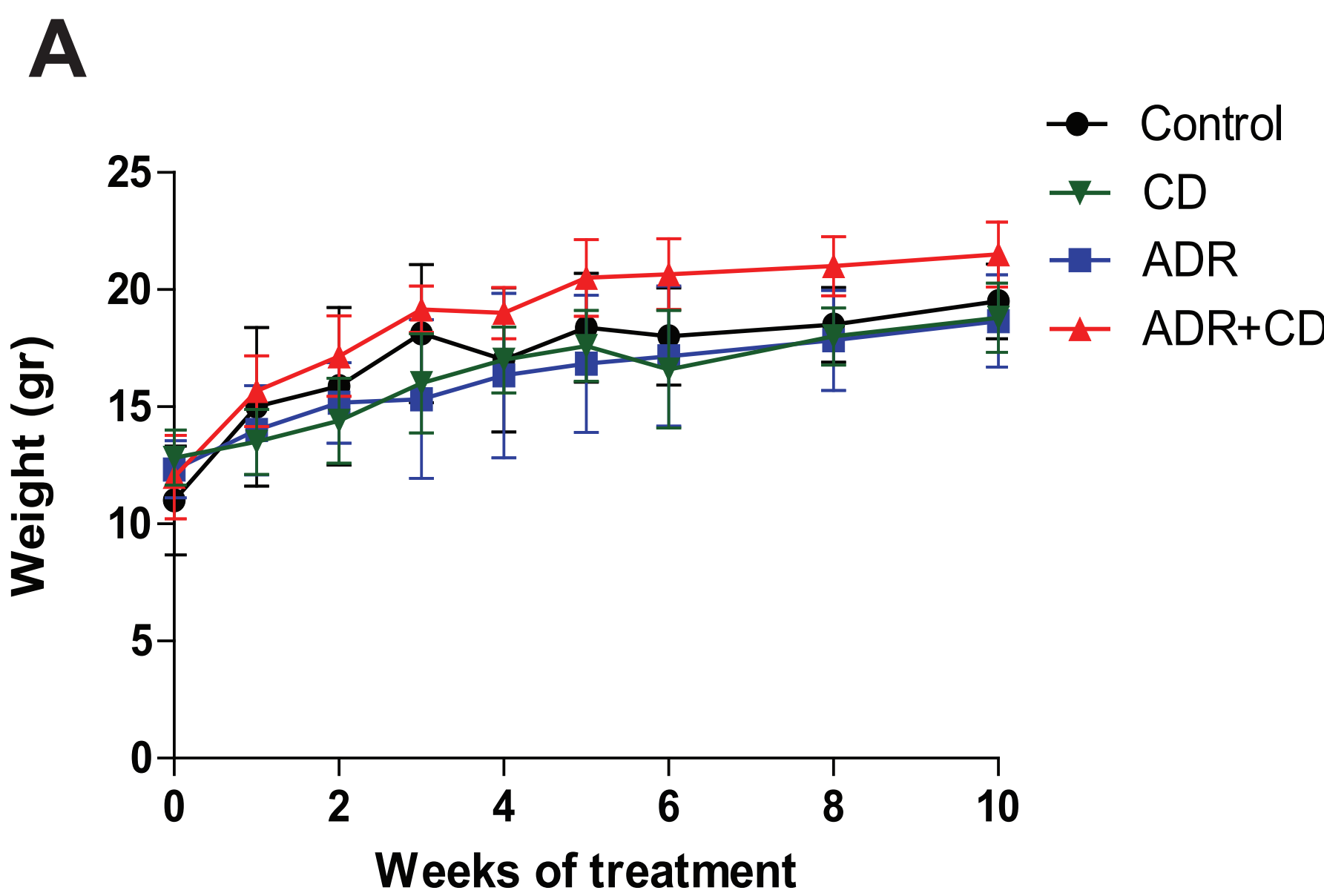


Figure 5. Body weight and renal function parameters in experimental model of FSGS *in vivo*. (A) Body weight had no changes between experimental groups of mice during the study. (B) Blood urine nitrogen (BUN) was significantly (*p<0.05, t-test) increased in the adriamycin treated group of mice (ADR) when compared to control and cyclodextrin treated only (CD) groups of mice. Cyclodextrin treatment improves the renal function of mice injected with adriamycin (ADR+CD). (C) Serum creatinine level is increased in adriamycin treated mice when compared to control, cyclodextrin only treated or adriamycin+cyclodextrin treated mice, but no significant changes were found.

SUMMARY

• Several lipid related genes are modulated in glomeruli from patients with FSGS

• Glomerular ApoL1 mRNA expression differentially correlates with lipid related genes in patients with two risk alleles when compared to no risk alleles

• In the adriamycin mouse model of FSGS cyclodextrin treatment is associated with a significant protection from albuminuria and with a trend to reduction of BUN and serum creatinine.

CONCLUSION

2-hydroxypropyl-beta-cyclodextrin protects podocytes in an experimental model of FSGS. As cyclodextrin was FDA approved for other indications (Niemann Pick Type C disease), it could be tested as a new therapy in patients affected by FSGS.

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