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Sterol-O-acyltransferase-1 has a role in kidney disease associated with diabetes and Alport Syndrome.

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Abstract

Defective cholesterol metabolism primarily linked to reduced ATP-binding cassette transporter A1 (ABCA1) expression is closely associated with the pathogenesis and progression of kidney diseases, including diabetic kidney disease and Alport Syndrome. However, whether the accumulation of free or esterified cholesterol contributes to progression in kidney disease remains unclear. Here, we demonstrate that inhibition of sterol-O-acyltransferase-1 (SOAT1), the enzyme at the endoplasmic reticulum that converts free cholesterol to cholesterol esters, which are then stored in lipid droplets, effectively reduced cholesterol ester and lipid droplet formation in human podocytes. Furthermore, we found that inhibition of SOAT1 in podocytes reduced lipotoxicity-mediated podocyte injury in diabetic kidney disease and Alport Syndrome in association with increased ABCA1 expression and ABCA1-mediated cholesterol efflux. *In vivo*, *Soat1* deficient mice did not develop albuminuria or mesangial expansion at 10–12 months of age. However, *Soat1* deficiency/inhibition in experimental models of diabetic kidney disease and Alport Syndrome reduced cholesterol ester content in kidney cortices and protected from disease progression. Thus, targeting SOAT1-mediated cholesterol metabolism may represent a new therapeutic strategy to treat kidney disease in patients with diabetic kidney disease and Alport Syndrome, like that suggested for Alzheimer's disease and cancer treatments.

Graphical Abstract

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Keywords

cholesterol; Alport Syndrome; diabetic kidney disease; SOAT1 Inhibitor; renal function; podocyte injury

INTRODUCTION

Emerging evidence demonstrates that renal lipid accumulation and lipotoxicity may contribute to kidney dysfunction¹⁻⁴. We and others recently demonstrated that glomerular accumulation of cholesterol, especially cholesterol ester (CE) accumulation occurs in experimental diabetic kidney disease (DKD) and Alport Syndrome (AS)⁵⁻⁹. These findings provide a rationale for therapeutic interventions targeting cellular cholesterol content¹⁰. Several inhibitors of CE transfer protein (CETP) effectively increase high density lipoprotein (HDL) cholesterol levels and have been developed for the treatment of dyslipidemia. However, many trials of these inhibitors have been discontinued due to safety concerns or lack of efficacy¹¹.

Cellular cholesterol homeostasis is highly regulated by various control mechanisms¹². The enzyme Sterol O-Acyltransferase (SOAT) previously known as acyl-CoA: cholesterol acyltransferase (ACAT), plays an important role in cellular cholesterol homeostasis. SOAT converts free cholesterol (FC) to CE which is then stored in lipid droplets (LDs)¹³. In mammals, two SOAT isoforms with different tissue expression patterns exist, SOAT1 and SOAT2¹⁴. While SOAT1 is ubiquitously expressed, SOAT2 is mainly expressed in fetal liver and intestinal cells but rarely in other tissues¹⁵⁻¹⁷. Previous studies described that using pharmacological or genetic strategies to block SOAT1 produces several beneficial effects in the treatment of Alzheimer's disease (AD) and atherosclerosis in cell culture and animal models¹⁸⁻²¹. Other studies suggested that inhibition of SOAT1 suppresses prostate cancer and glioblastoma cell growth^{22, 23}.

In previous studies, we demonstrated that the expression of ATP-binding cassette A1 (ABCA1), an important regulator of reverse cholesterol transport, is decreased in several glomerular disorders of non-metabolic and metabolic origin, including AS and DKD^{5, 8}.

Additionally, these studies revealed a positive correlation between renal CE content and blood urine nitrogen (BUN) in experimental models of DKD⁵, suggesting that preventing CE accumulation may prove beneficial to prevent renal lipid accumulation and improve renal disease progression. We have demonstrated that pharmacological induction of ABCA1 with preclinical ABCA1 inducers prevents the accumulation of CE in the kidney cortices and protects from DKD⁵. However, whether altering the FC/EC ratio by inhibition of SOAT1 would benefit renal function and disease progression remains unknown. This study is aimed at investigating whether blocking cholesterol esterification via SOAT1 inhibition prevents renal CE accumulation and may treat renal disease associated with DKD and AS.¹⁴

RESULTS

SOAT1 inhibition reduces the CE content in human podocytes by upregulating ABCA1-mediated cholesterol efflux.

We previously described that inhibition of SOAT1 in combination with impaired cholesterol efflux causes FC accumulation leading to podocyte injury and loss⁷. We also demonstrated that CE accumulation occurs in experimental models of DKD^{5, 6}, suggesting that both FC and/or CE accumulation may contribute to podocyte injury in renal disease. Since SOAT1 is an important enzyme regulating CE synthesis²⁴, we investigated the role of SOAT1 in human podocytes. Human podocytes were treated with a SOAT1 inhibitor (SI, 4 μ M) or DMSO (Ctrl) for 48 hours, followed by incubation with [¹⁴C]-oleic acid. SOAT1 activity was measured by assessment of [¹⁴C] incorporation in the esterified cholesterol pool. We found that SOAT1 activity was significantly decreased in the SI treated podocytes (Supplemental Figure 1). As shown, SI treatment significantly reduced lipid content, as quantified by Nile red staining (Figures 1a, 1e), in human podocytes. Because LDs are mainly composed of CE and triglycerides (TG), we next evaluated the CE and TG content of SI-treated podocytes. As expected, CE (Figure 1b) and TG (Figure 1c) content were both significantly decreased in SI-treated podocytes. SI treatment did not have a cytotoxic effect on human podocytes (Figure 1d). Taken together, our data demonstrate that SOAT1 plays a critical role in regulating CE synthesis and LD formation in human podocytes.

We previously demonstrated that ABCA1 is a susceptibility factor contributing to cholesterol accumulation, glomerular injury and renal disease progression in AS and DKD. However, ABCA1 deficiency⁵ or SOAT1 inhibition (Figure 1d) alone is not sufficient to cause podocyte cytotoxicity. In additional studies, we demonstrate that SI treatment in the setting of ABCA1 deficiency in podocytes causes podocyte injury⁷. These observations led us to hypothesize that SOAT1 inhibition reduces cellular CE levels but does not increase FC accumulation in human podocytes (Supplemental Figure 2), possibly through a mechanism by which SI increases ABCA1 expression in podocytes. We therefore evaluated ABCA1 mRNA expression in SI-treated podocytes and found that ABCA1 expression is significantly increased in SI-treated when compared with vehicle-treated human podocytes (Figure 1f and Supplemental Figure 3). Supporting this observation, we also found significantly increased ABCA1-mediated cholesterol efflux in SI-treated human podocytes when compared to the vehicle-treated group (Figure 1g). These data suggest that SI not only reduces the CE content, but also prevents FC accumulation in human podocytes by increasing ABCA1

expression and APOA1-mediated cholesterol efflux. However, the exact mechanism by which SOAT1 inhibition protects podocytes from injury in DKD warrants further investigation.

SOAT1 inhibition protects podocytes from DKD sera-induced cytotoxicity through modulation of ABCA1 expression.

We previously described that ABCA1 expression is reduced in podocytes exposed to the sera from type 2 diabetic patients (T2D) with progressive kidney disease (P sera) and is associated with increased lipid droplet content and cytotoxicity⁵. To test if SOAT1 expression is upregulated in P sera exposed podocytes, normal human podocytes were exposed to P sera or to sera from T2D patients with non-progressive kidney disease (NP sera) as control for 48 hours. Quantitative real-time PCR showed increased SOAT1 expression in podocytes exposed to P sera compared to NP sera (Figure 2a). In order to understand if SI treatment can protect podocytes from P sera-induced cytotoxicity, normal human podocytes were exposed to P sera or NP sera for 48 hours in the presence of SI or vehicle control (Ctrl). Quantitative real-time PCR was performed to evaluate ABCA1 expression. We found that SI treatment protects podocytes from the P sera-induced reduction in ABCA1 expression (Figure 2b). More importantly, SI treatment also reduced P sera-induced cytotoxicity (Figure 2c). To further evaluate if this protection is mediated in an ABCA1-dependent manner, siCO and siABCA1 podocytes were used. ABCA1 knockdown in siABCA1 podocytes was confirmed by Western blot analysis (Supplemental Figure 4). siCO and siABCA1 podocytes were treated with P sera in the presence of SI or vehicle control. We observed that SI treatment of siCO decreased cytotoxicity by approximately 70%, while cytotoxicity in siABCA1 was decreased by only 40% (Figure 2d). SI treatment also protected podocytes from apoptosis in presence of P sera (Supplemental Figure 5). These results suggest that the protective effect of SI treatment on P sera-induced cytotoxicity is partially mediated by ABCA1.

Soat1 knockout mice have normal renal function.

To investigate whether SOAT1 deficiency affects renal function *in vivo*, *Soat1* knockout mice (*Soat1*^{-/-})²⁵ were analyzed at 10–12 months of age. As expected, SOAT1 expression was undetectable in kidney cortices of *Soat1*^{-/-} mice (Figure 3a). However, no change in the renal cholesterol content was observed in *Soat1*^{-/-} mice compared to WT mice (Figure 3b and Supplemental Figure 6). Interestingly, ABCA1 expression was found to be increased in *Soat1*^{-/-} mice (Figure 3c), which might explain why no change in renal cholesterol content was detected. This observation was further confirmed by Oil Red O staining, which did not detect any difference in the renal lipid content between WT and *Soat1*^{-/-} mice (Figures 3d, 3e). Finally, SOAT1 deficiency did not affect body weight, fasting plasma glucose, albumin/creatinine ratios, BUN (Figure 3f) or mesangial expansion (Figures 3g, 3h). These data indicate that genetic deletion of *Soat1* in mice does not affect renal cholesterol content or renal function, although it may negatively affect liver function (Supplemental Table 1).

Soat1 deficiency prevents renal lipid accumulation, protects from proteinuria and preserves renal histology in *db/db* mice.

We previously demonstrated that renal CE accumulation is associated with disease progression in experimental DKD⁵. However, whether CE accumulation contributes to DKD progression remains unclear. We therefore investigated if *Soat1* deficiency could prevent renal CE accumulation and protect renal function in experimental DKD (*db/db* mice). Double-homozygous *db/db;Soat1*^{-/-} mice were generated by breeding double-heterozygous *db/+;Soat1*^{+/-} littermates. At 16 weeks of age (time of sacrifice), albuminuria decreased in *db/db;Soat1*^{-/-} mice when compared to diabetic control mice (*db/db;Soat1*^{+/+}), but it did not reach statistical significance when using Bonferroni correction (defined with a critical significance of $P < 0.016$) (Figure 4a). *db/db;Soat1*^{-/-} mice were also characterized by a decreased CE content in kidney cortices (Figure 4b, Supplemental Figure 7), increased ABCA1 expression in glomeruli (Figure 4c) and decreased mesangial expansion (Figures 4d, 4e) compared to *db/db;Soat1*^{+/+} mice. WT1 immunofluorescence staining was utilized to quantify podocyte number per glomerular cross section. We found that *db/db;Soat1*^{-/-} mice have significantly preserved podocyte numbers per glomerular section when compared to *db/db;Soat1*^{+/+} mice (Figures 4f, 4g), indicating that SOAT1 deficiency prevents podocyte loss. Finally, using transmission electron microscopy (TEM), we observed that *db/db;Soat1*^{-/-} mice have a higher number of foot processes per μm of glomerular basement membrane (GBM) when compared to *db/db;Soat1*^{+/+} mice (Figures 4h, 4i), indicating that SOAT1 deficiency prevents podocyte foot process effacement. However, we did not observe a beneficial effect of SOAT1 deficiency in *db/db* mice with regard to body weight, blood glucose levels, insulin sensitivity and serum lipids level (Supplemental Figure 8), as well as with regard to glomerular surface area and GBM thickness (Supplemental Figure 9) when compared to *db/db;Soat1*^{+/+} littermates. Taken together, these data suggest that *Soat1* deficiency prevents CE accumulation, protects from proteinuria, and preserves renal histology in experimental models of DKD.

SOAT1 inhibition improves renal function in experimental Alport Syndrome.

We previously described that Col4a3 knockout mice, a mouse model of AS, are characterized by renal CE accumulation⁸. To determine SOAT1 activity in primary podocytes isolated from AS mice, glomeruli from 9-week-old WT and AS mice were isolated and primary podocyte cultures were established²⁶. SOAT1 activity was significantly increased in primary AS podocytes when compared to WT control podocytes (Figure 5a). To further evaluate the impact of *Soat1* inhibition in podocytes of AS mice, primary WT and AS podocytes were treated with SI or vehicle. We found that SI treatment significantly decreases apoptosis in SI-treated compared with vehicle-treated AS podocytes (Figure 5b). To determine whether pharmacological inhibition of *Soat1* would improve renal function *in vivo*, AS mice were injected daily i.p. with SI (15mg/kg) starting at 4 weeks of age for 4 weeks. Mice were sacrificed at 8 weeks of age. Albumin-to-creatinine ratio (ACR) decreased in SI-treated AS mice when compared with AS mice, but it did not reach statistical significance when using Bonferroni correction (Figure 5c). We detect a significant decrease in BUN, and serum creatinine in SI-treated AS mice when compared with AS mice (Figures 5d–e). Unlike what we observed in *Soat*^{-/-} mice, ALT concentrations were not negatively affected by SI in mice with AS (162 ± 5 U/L in AS+Vehicle versus 61.67 ± 48.84 U/L in AS

+SI). Administration of SI also significantly prevented body weight loss during renal disease progression in AS mice (Figure 5f) and reduced mesangial expansion (Figures 6a, 6b). To investigate if SI treatment prevents lipid accumulation in AS mice, the CE content of kidney cortices was determined and Oil Red O (ORO) staining of kidney sections was performed. As expected, we found a decreased number of ORO positive glomeruli in SI-treated AS mice when compared with vehicle-treated AS mice (Figures 6c, 6d) and a decreased CE content in kidney cortices (Figure 6e, Supplemental Figure 10). Picrosirius Red staining of kidney sections confirmed increased fibrosis in AS mice, while SI treatment significantly decreased glomerular and interstitial fibrosis in SI-treated AS mice (Figures 6f, 6g). Importantly, electron microscopy analysis revealed significant protective effects on podocyte foot process effacement and glomerular basement membrane thickness in SI-treated AS mice compared with untreated AS mice (Figure 6h–j). In a previous study, we demonstrated reduced Abca1 expression in glomeruli of AS mice⁸. We therefore investigated whether SI treatment would increase ABCA1 expression in the AS mice. Quantitative real-time PCR showed increased ABCA1 expression in glomeruli of SI-treated AS mice compared with untreated AS mice (Supplemental Figure 11), further supporting the hypothesis that SI treatment protects podocytes from injury by altering the renal CE content, thus improving proteinuria, fibrosis, mesangial expansion and renal function.

DISCUSSION

With this study, we demonstrate for the first time that targeting SOAT1-mediated cholesterol metabolism benefits renal function and prevents disease progression in experimental models of DKD and AS. We further define the mechanism by which inhibition of SOAT1 reduces CE accumulation and increases ABCA1/APOA1-mediated cholesterol efflux thus protecting podocytes from injury and lipotoxicity.

It has been established that accumulation of excess FC is toxic to cells^{27, 28}, however, to date a role for CE in cellular toxicity has not been established. In this study, we demonstrate that treatment of human podocytes with SI does not cause podocyte cytotoxicity but reduces CE, TG and lipid content by increasing ABCA1/APOA1-mediated cholesterol efflux (Figure 1). Interestingly, *in vivo*, *Soat1* deficiency is not sufficient to change the cholesterol content of kidney cortices, to cause proteinuria or mesangial expansion in *Soat1*^{-/-} mice (Figure 3), suggesting that compensatory mechanisms might regulate proper cholesterol homeostasis *in vivo*. Furthermore, the observation that the combined deficiency of *Abca1* and *Soat1* in *Abca1*^{fl/fl}; *Soat1*^{-/-} mice (DKO) leads to FC accumulation in kidney cortices in the absence of the development of albuminuria or mesangial expansion⁵ suggests that FC accumulation *in vivo* is not sufficient to cause podocyte injury and proteinuria. Interestingly, we also demonstrate a strong positive correlation between the CE content of kidney cortices and BUN in mice with glomerular disease^{5, 8}, leading us to hypothesize that *in vivo* CE accumulation rather than FC accumulation might contribute to cellular toxicity and renal disease progression in glomerular disease.

In order to test if converting CE to FC by the inhibition of SOAT1 protects podocytes from injury and preserves renal function and disease progression, we performed a series of *in vitro* (Figure 2) and *in vivo* (Figures 4, 5, 6) experiments. *In vitro*, we investigated the effect

of SI on podocytes that were treated with the sera from diabetic patients (T2D) with progressive kidney disease (P sera). SI treatment prevented the reduction of ABCA1 expression and cytotoxicity in normal human podocytes exposed to P sera. A weaker effect was observed in siABCA1 compared with siCO podocytes (Figure 2d), indicating that SI protects podocytes from P sera-induced cytotoxicity in an ABCA1-dependent mechanism. In support of these results, a recent study using murine peritoneal macrophages demonstrated that lack of SOAT1 leads to FC accumulation and increases cholesterol efflux²⁹. Another supportive study showed that in mouse fibroblasts, APOA1 mobilizes intracellular cholesterol for ABCA1-mediated release from the compartment that is under the control of SOAT1³⁰. Interestingly, additional studies revealed that blocking SOAT1 by using a SOAT1 inhibitor increases the ER regulatory sterol pool, and leads to downregulation of HMG-CoA reductase and sterol regulatory element binding protein processing in macrophages and in CHO cells^{31, 32}. Shibuya *et al.* found that blocking SOAT1 in cultured microglia by gene knockout or by the SOAT1 inhibitor K604 caused increases in autophagy and in TFEB-mediated lysosome biogenesis in an mTOR-independent manner³³. Thus, while we have shown that increasing ABCA1 is one of the mechanisms of podocyte protection by SOAT1 inhibition, the observed protective effect of SOAT1 inhibition may be possible through other systemic and local metabolic pathways involving lipid synthesis and autophagy.

Our results demonstrate that the expression and activity of SOAT1 is elevated in experimental models of DKD (Figure 2a) and AS (Figure 5a) and that deletion/inhibition of SOAT1 could represent a new therapeutic strategy for the treatment of patients with DKD and AS. To investigate the effect of SOAT1 inhibition, we used mouse models of metabolic (db/db mice) and non-metabolic (AS mice) glomerular disease. We first generated db/db;Soat1^{-/-} mice. Consistent with our *in vitro* findings, at 16 weeks of age, SOAT1 deficiency in db/db;Soat1^{-/-} mice reduced albuminuria and foot process effacement, reduced the CE/TC content in kidney cortices, improved the mesangial expansion score and protected from podocytes loss during DKD progression when compared to db/db;Soat1^{+/+} mice (Figure 4). Several studies have investigated the role of cellular lipid accumulation on tubulointerstitial injury and fibrosis³⁴⁻³⁷. Kang *et al* demonstrated that the expression of key enzymes and regulators of fatty acid oxidation (FAO) is decreased, while intracellular lipid deposition is increased in humans and in mouse models with tubulointerstitial fibrosis³⁷. Others demonstrated that mice fed on a cholesterol/fat rich diet are characterized by increased accumulation of free cholesterol and phospholipids within multilamellar organelles of lysosomal origin in renal proximal tubular epithelial cells³⁴. Taken together, these studies suggest an important role of cellular cholesterol accumulation in obesity-related renal changes.

To test if inhibition of SOAT1 also improves renal function in non-metabolic glomerular disorders, Col4a3 knockout mice (AS mice) were treated with a SI. Similarly to what we observed in db/db;Soat1^{-/-} mice, SI significantly reduces renal CE content and protects from the development of albuminuria, renal failure and fibrosis in AS mice (Figures 5, 6). More importantly, we demonstrate that these changes are associated with increased glomerular ABCA1 expression in db/db;Soat1^{-/-} mice (Figure 4c) and SI-treated AS mice (Supplemental 11) when compared with db/db;Soat1^{+/+} and untreated AS mice, respectively. Whether the upregulation of ABCA1 by SI is direct or indirect remains to be established.

It has been suggested that pharmacological inhibition and genetic deletion can have similar outcomes in various diseases^{38, 39}. However, in some cases genetic deletion and pharmacological inhibition can elicit markedly different phenotypes while targeting the same protein⁴⁰. While small molecules typically do not alter the expression of their target protein but rather protein function/activity, gene knockout results in complete loss of the target protein, which can disrupt protein complexes or impair functional domains that would be unaffected by a drug⁴⁰. While we found effective prevention of the renal phenotype in *db/db* mice with a genetic deletion of *Soat1*, we were not able to detect improvement in ACR or cholesterol ester content following SI treatment when used at the same dose as in the AS model (data not shown). Therefore, further pharmacokinetic studies may be needed to advance the use of SOAT1 inhibitors into clinical applications. SI was also found to be beneficial in reducing atherosclerosis⁴¹, improving amyloid pathology, and ameliorating symptoms in experimental models of Alzheimer's disease^{18, 19}. SI (pactimibe sulfate, CS-505) has been studied for the treatment of carotid artery disease, coronary heart disease, and atherosclerosis in phase II and/or phase III clinical trials ([NCT00151788](#), [NCT00185042](#), [NCT00185146](#)). Although these compounds are safe, they are mostly found not to be effective. Thus, a repurposing strategy of SI for other indications should be considered. However, the observation that currently available SIs are very hydrophobic compounds may pose some concern. It would therefore be desirable to develop new SIs that are more soluble and safer to deliver, or alternatively, to further develop ABCA1 inducers that we have also found to reduce CE content in experimental DKD⁵.

Our study has several limitations. First, we do not have *in vivo* proof that the protection offered by SOAT1 inhibition occurs only through the modulation of ABCA1. It is possible that other pathways are involved. In addition, some of the experiments included only 5 mice per group; thus, some comparisons did not have the power to show significant differences.

In conclusion, our study provides evidence that CE, not FC accumulation, induces lipotoxicity and contributes to renal disease in experimental models of metabolic and non-metabolic glomerular disorders. We demonstrate that strategies that are aimed at preventing renal CE accumulation or at reducing renal CE content effectively improve renal function in experimental models of DKD and AS. Finally, we show that the mechanism by which SI protects podocyte from injury is, at least in part, through modulation of ABCA1 expression and induction of ABCA1/APOA1-mediated cholesterol efflux. We propose that SI may represent a novel therapeutic strategy for the treatment of patients with DKD and AS and potentially for the treatment of other disorders in which altered renal cholesterol metabolism contributes to podocyte injury.

METHODS

Animal studies

Soat1 knockout mice²⁵ (C57BL/6J background) were gifted from Dr. Ta-Yuan Chang (Dartmouth, Hanover, NH). *Soat1*^{-/-} mice were sacrificed at 10–12 months of age. *Soat1*^{-/-} mice are fertile and grow normally. They develop narrow eye fissures and hair loss as described in detail by others^{25, 42}.

db/db;Soat1^{-/-} mice were generated by breeding of *db/+* (C57BL6 background, Jackson Laboratory, stock#00697) with *SOAT1^{-/-}* mice followed by breeding of double heterozygous littermates. Double homozygous *db/db;Soat1^{-/-}* were generated and analyzed with *db/+;Soat1^{+/+}*, *db/db;Soat1^{+/+}* littermates as controls at 16 weeks of age. Both male and female mice were included in all groups and in all experiments.

Col4a3 knockout mice were used as a model of AS. Mice are in a 129X1/SvJ background and were purchased from Jackson Laboratory (129-Col4a3 *tm1Dec/J*, stock#002908). 4-week-old AS mice were daily injected with SOAT1 inhibitor (SI) as previously described²². SI was dissolved in DMSO, then diluted with PBS containing 1% Tween-80. SI was administered via daily intraperitoneal injection for 4 weeks at dose of 15mg/kg. This dose was selected based on previous animal studies of these inhibitors, and the indicated IC 50 of Sandoz 58–035 of ~3.3μM^{43–47}. Mice were sacrificed at 8 weeks and analyzed as described in the Methods of Supplemental Material.

Podocyte cell culture

Human podocytes were cultured at 33°C under permissive conditions in RPMI culture medium containing 10% FBS and 1% penicillin/streptomycin and 0.01mg/ml recombinant human insulin, 0.0055 mg/ml human transferrin (substantially iron-free), and 0.005 μg/ml sodium selenite⁴⁸. Human podocytes were then thermoshifted and differentiated for 14 days at 37°C in RPMI medium 10% FBS and 1% penicillin/streptomycin. ABCA1 siRNA knockdown (siABCA1) and non-targeting siRNA control (siCO) were generated as previously described⁷. For treatment with patient sera, at day 11 of differentiation, human podocytes were serum starved for 24 hours, followed by treatment with 4% patient sera in combination with Sandoz 58–035 (4μM, Sigma) or DMSO for 48 hours. For treatment without patient sera, at day 12 of differentiation, human podocytes were incubated with SI or DMSO for 48 hours.

Additional information about Methods can be found in the Supplemental Material.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

DISCLOSURE

AF and SM are inventors on pending or issued patents (PCT/US11/56272, PCT/US12/62594, PCT/US2019/041730, PCT/US2019/032215, PCT/US13/36484 and PCT 62/674,897) aimed to diagnosing or treating proteinuric kidney diseases. They stand to gain royalties from their future commercialization of these patents. AF is Vice-President of L&F Health LLC and is consultant for ZyVersa Therapeutics, Inc. ZyVersa Therapeutics, Inc has licensed worldwide rights to develop and commercialize hydroxypropyl-beta-cyclodextrin from L&F Research for the treatment of kidney disease. SM is a consultant for Kintai Therapeutics, Inc and holds equity interest in L&F Research. AF and SM are supported by Hoffman-La Roche and by Boehringer Ingelheim.

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Figure 1. SOAT1 inhibitor (SI) treatment of human podocytes reduces cholesterol ester (CE), triglyceride (TG) and lipid droplet (LD) content by increasing ABCA1 expression and ABCA1-mediated cholesterol efflux.

Human podocytes were treated with SOAT1 inhibitor (SI, Sandoz 58-035, 4 μ M) or DMSO (Ctrl) for 48hrs. (a) Bar graph analysis showing the quantification of the images shown in (e). Lipid content was measured via Nile red fluorescent intensity per cell (n=15–20), (b) Cholesterol content represented as CE/TC (%) (n=5), (c) TG content (n=5). (d) Bar graph analysis showing cytotoxicity normalized to viability (n=4–5). (e) Representative images (original magnification x20) showing Nile red (green) and DAPI (blue) staining. (f) Quantitative real-time PCR analysis of ABCA1 expression in DMSO-treated (Ctrl) or SI-treated (SI) human podocytes (n=5). (g) Quantification of the percentage of cholesterol effluxed via the ABCA1 transporter in DMSO-treated (Ctrl) or SI-treated (SI) human podocytes (n=5). Kruskal-Wallis test (g), or Mann-Whitney test (all other panels).

Figure 2. SOAT1 inhibitor (SI) treatment increases ABCA1 expression and protects podocytes from injury.

(a) Quantitative real-time PCR analysis of SOAT1 expression in podocytes that were exposed to NP and P patient sera from T2D patients (n=4). (b) Quantitative real-time PCR analysis of ABCA1 expression in DMSO-treated (Ctrl) or SI-treated normal human podocytes that were exposed to NP sera and P sera (n=5). (c) Bar graph analysis showing cytotoxicity normalized to viability in DMSO-treated (Ctrl) or SI-treated normal human podocytes that were exposed to NP and P patient sera (n=6–7). (d) Bar graph analysis showing cytotoxicity normalized viability in DMSO-treated or SI-treated siCO and siABCA1 podocytes that were exposed to P sera (n=5). Mann-Whitney test (a) or Kruskal-Wallis test (all other panels).

Figure 3. *Soat1* knockout mice do not develop albuminuria or mesangial expansion at 10–12 months of age.

(a) Quantitative real-time PCR analysis of *SOAT1* expression in kidney cortices of WT and *Soat1*^{-/-} mice (n=6–7). (b) Bar graph analysis showing the quantification of CE/TC (%) from kidney cortices of WT and *Soat1*^{-/-} mice at 10–12 months of age. (c) Quantitative real-time PCR analysis of *ABCA1* expression in kidney cortices of WT and *Soat1*^{-/-} mice (n=8–9). (d, e) Representative images (d) and bar graph quantification (e) of Oil red O staining of kidney cortices (scale bar: 20 μ m, n=7). (f) Summary of body weight, fast plasma glucose, albumin/creatinine, BUN in WT and *Soat1*^{-/-} mice (n=8–9). (g, h) Representative images (g) and bar graph quantification of the mesangial expansion score (h) determined by PAS staining (scale bar: 20 μ m, n=5–6). Mann-Whitney test.

Figure 4. *Soat1* deficiency improves renal function in diabetic mice (*db/db*).

Mice were sacrificed and analyzed at 16 weeks of age. (a) *db/+;Soat1^{+/+}*, *db/db;Soat1^{+/+}*, and *db/db;Soat1^{-/-}* littermates were analyzed for albumin-to-creatinine ratios (ACR) determined at 16 weeks of age (n=5–9). (b) Bar graph analysis showing the kidney cortices cholesterol content represented as CE/TC(%) in *db/+;Soat1^{+/+}*, *db/db;Soat1^{+/+}*, and *db/db;Soat1^{-/-}* mice (n=5–10). (c) Quantitative real-time PCR analysis of ABCA1 expression in glomeruli of *db/+;Soat1^{+/+}*, *db/db;Soat1^{+/+}*, and *db/db;Soat1^{-/-}* mice (n=5–8). (d,e) Representative images of PAS staining (d) and bar graph analysis of the mesangial expansion score (e) in *db/+;Soat1^{+/+}*, *db/db;Soat1^{+/+}*, and *db/db;Soat1^{-/-}* (scale bar: 20 μm) (n=5–6). (f, g) Representative images of WT1 staining (f) and bar graph analysis of the number of WT1-positive cells per glomerular cross section (g) in *db/+;Soat1^{+/+}*, *db/*

db;Soat1^{+/+}, and *db/db;Soat1^{-/-}* mice (scale bar: 25 μ m) (n=5–6). **(h, i)** Representative images of TEM images **(h)** and bar graph quantification of the number of foot processes effacement (blue arrowheads) per μ m of GBM **(i)** in *db/+;Soat1^{+/+}*, *db/db;Soat1^{+/+}*, and *db/db;Soat1^{-/-}* mice (scale bars: 500 nm) (n=5–6). Kruskal-Wallis test.

Figure 5. SOAT1 Inhibitor (SI) improves renal function in a mouse model of AS.

(a) Bar graph analysis showing SOAT1 activity in immortalized primary podocytes isolated from WT and AS mice (n=9–10). (b) Immortalized primary podocytes of WT and AS mice treated with SI or DMSO for 48hr. Bar graph analysis showing apoptosis normalized to viability (n=6). (c–f) 4-week-old AS mice received daily i.p injected of SI (15mg/kg) for 4 weeks. Three groups of mice were analyzed: WT (n=7), AS (n=7) and AS+SI (n=5–6) mice. (c) Bar graph analysis showing the quantification of albumin-to-creatinine ratios (ACR), (d) blood urea nitrogen (BUN), (e) serum creatinine levels, (f) body weight (BW). Mann-Whitney test (a) or Kruskal-Wallis test (all other panels).

Figure 6. SOAT1 Inhibitor (SI) improves renal histology in a mouse model of AS. 4-week-old AS mice received daily i.p injected of SI (15mg/kg) for 4 weeks. Three groups of mice were analyzed: WT (n=5–8), AS (n=5–8) and AS+SI (n=5–6) mice. (a, b) Representative images of PAS staining (a) and bar graph analysis of the mesangial expansion score (b) in WT, AS and AS+SI mice (scale bar: 20 μ m). (c, d) Representative images (c) quantification (d) of Oil red staining of kidney cortices (scale bar: 20 μ m). (e) Kidney cortices cholesterol content represented as CE/TC (%). (f, g) Representative images (f) and bar graph quantification of glomerular and interstitial fibrosis by Picrosirius Red staining (g) (scale bar: 20 μ m). (h–j) Representative images of transmission electron micrograph (TEM) (h) and bar graph quantification of foot process effacement (blue arrowheads) (i) and glomerular basement membrane thickness (orange arrowheads) (j) (scale bar: 500 nm). Kruskal-Wallis test.