Hepatocyte nuclear factor 4α regulates megalin expression in proximal tubular cells

Shota Sasaki\textsuperscript{a}, Ayami Hara\textsuperscript{a}, Masakiyo Sakaguchi\textsuperscript{b}, Masaomi Nangaku\textsuperscript{c}, Yusuke Inoue\textsuperscript{a,d,\*}

\textsuperscript{a} Division of Molecular Science, Graduate School of Science and Technology, Gunma University, Kiryu, Gunma 376-8515, Japan
\textsuperscript{b} Department of Cell Biology, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama 700-8558, Japan
\textsuperscript{c} Division of Nephrology and Endocrinology, the University of Tokyo Hospital, Tokyo 113-8655, Japan
\textsuperscript{d} Division of Molecular Science, Graduate School of Science and Technology, Gunma University, Kiryu, Gunma 376-8515, Japan.

\* Corresponding author at: Division of Molecular Science, Graduate School of Science and Technology, Gunma University, 1-5-1 Tenjin-cho, Kiryu, Gunma 376-8515, Japan.

E-mail address: yinoue@gunma-u.ac.jp (Y. Inoue).

ABSTRACT

Hepatocyte nuclear factor 4α (HNF4α) is a member of the nuclear receptor superfamily and upregulates expression of many genes in the liver, pancreas, small intestine, and colon. HNF4α is also highly expressed in proximal tubular epithelial cells (PTECs) in kidney. PTECs reabsorb various substances through transporters, ion channels, and receptors, but the target genes for HNF4α in PTECs have not been investigated in detail. In the present study, we aimed to identify novel HNF4α target genes that are highly expressed in PTECs. Expression of many solute carrier transporter genes was upregulated by HNF4α in human PTEC-derived HK-2 cells. Notably, expression of megalin (LRP2), an endocytic receptor of various molecules involved in development and progression of chronic kidney disease (CKD), was strongly induced by HNF4α, and the transactivation potential of the megalin promoter was dependent on HNF4α expression. Moreover, HNF4α was found to directly bind to an HNF4α binding site near the transcription start site in the megalin gene. These results indicate that HNF4α plays an important role in maintaining reabsorption and metabolism in PTECs by positive regulation of several solute carrier transporter and megalin genes at the transcriptional level.

1. Introduction

Hepatocyte nuclear factor 4α (HNF4α), an orphan member of nuclear receptor superfamily, is highly expressed in epithelial cells in liver, pancreas, small intestine, colon, and kidney, and HNF4α is essential for maintenance of the homeostasis of these tissues [1,2]. HNF4α binds to approximately 40% of the promoter region of the genes expressed in human hepatocytes and pancreatic islets [3], indicating that HNF4α is a strong regulator in the liver and pancreatic. Since Hnf4a-null mice results in embryonic lethality [4], tissue-specific Hnf4a-null mice were generated in hepatocytes, pancreatic β cells, and intestinal epithelial cells. Based on many studies using liver-specific Hnf4a-null mice, hepatic HNF4α was found to be a central regulator for hepatocyte differentiation and function through direct regulation of many liver-specific genes [5–9]. In addition, pancreatic β cell-specific Hnf4a-null mice impaired glucose-stimulated insulin secretion [10]. Mutations in HNF4α gene related to maturity-onset diabetes of the young 1 (MODY1) [11], suggesting that pancreatic β cell-specific Hnf4α-null mice exhibited the similar phenotype as MODY1. Also, intestinal epithelial cell-specific Hnf4α-null mice were liable to get inflammatory bowel disease (IBD) [12]. Thus, HNF4α plays an essential role in maintenance of specific function in these tissues, but kidney-specific Hnf4a-null mice have not generated yet.

In kidney, HNF4α is highly expressed in proximal tubular epithelial cells (PTECs) [13,14]. The proximal tubule is a part of the nephron of the kidney, and the main function of the proximal tubule is reabsorption of many substances such as water, glucose, potassium and sodium ions, phosphate, amino acids, and proteins filtrated in glomerulus. PTECs play an important role in renal injury and repair in acute kidney injury (AKI) and chronic kidney disease (CKD) progression [15–17]. Many transporters such as solute carrier (SLC) transporters and ATP-binding cassette transporters are expressed in the proximal tubule to reabsorb and excrete biomolecules. For example, sodium glucose co-transporter 2 (SGLT2), as known SLC5A2, is expressed in the proximal tubule, and Sglt2-deficient mice exhibit glycosuria and decreased glucose reabsorption [18]. Thus, many SGLT2 inhibitors are on the market.

Abbreviations: HNF4α, hepatocyte nuclear factor 4α; CKD, chronic kidney disease; PTECs, proximal tubular epithelial cells

Received 31 October 2018; Received in revised form 27 November 2018; Accepted 28 November 2018

2405-5808/ © 2018 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY license (http://creativecommons.org/licenses/BY/4.0/).
for type 2 diabetic medications. Moreover, several studies were reported that HNF4α regulates expression of the transporters. Expression of Slc6a19 gene, the neutral amino acid transporter in enterocytes, is transactivated by HNF4α and HNF1α [19]. HNF4α was also shown to bind to the promoter regions of Slc22a1, Slc22a6, and Slc22a8 genes in rat kidney [20], indicating that HNF4α could play a central regulator of the transporters in the proximal tubule. However, the detailed regulation of the proximal tubule-enriched genes including the transporters by HNF4α remains poorly unexplained.

In the present study, we investigated to identify novel HNF4α target genes that are highly expressed in PTECs by overexpression of HNF4α in human HK-2 cells that express many characteristics of PTECs [21]. We found that expression of many transporters was induced by HNF4α, but expression of megalin (known as LRP2), a receptor for a large number of ligands, was strongly induced by HNF4α. Promoter activity of megalin gene was dependent on an HNF4α binding site and HNF4α expression, and HNF4α directly bound to the HNF4α binding site. Because megalin is involved in CKD development and progression, these finding may contribute to a detailed understanding of CKD development and progression through direct regulation of megalin expression by HNF4α.

2. Experimental procedures

2.1. Cell culture

HK-2 and HEK293T cells were cultured at 37°C in Dulbecco’s modified Eagle’s medium (Wako) containing 10% fetal bovine serum (HyClone) and 100 units/ml penicillin/streptomycin (Thermo Fisher Scientific).

2.2. Construction of HNF4α expression plasmid and transient transfection

Full-length of human HNF4α cDNA was cloned into EcoRI and BamHI sites of pCMVIR [22] and pEBMulti-Hygro vectors (Wako). pCMVIR/HNF4α or empty plasmids were transfected into HK-2 cells cells using Fugene HD (Roche) and HEK293T cells using polyethyleneimine Max (Polyscience) as transfection reagents. After 48 h, cells using Fugene HD (Roche) and HEK293T cells using polyethyleneimine Max (Polyscience) as transfection reagents. After 48 h, promoter activities were measured using Dual-Glo Luciferase Assay System (Promega).

2.6. Western blot

Whole cell lysates from cultured cell lines were also prepared as described previously [8]. The samples were diluted with Laemmli sample buffer, incubated at 65°C for 15 min, fractionated by 10% SDS-polyacrylamide gel electrophoresis. The gels were transferred onto a PVDF membrane (GE healthcare). The membrane was incubated for 1 h with PBS containing 0.1% Tween 20% and 5% skim milk, and then incubated for 1 h with anti-HNF4α (Perceus Proteomics) and anti-γ-tubulin (Sigma-Aldrich) antibodies. After washing, the membrane was incubated for 1 h with horseradish peroxidase-conjugated secondary antibodies (Cell Signaling Technology), and the reaction product was visualized using Western Lightning Ultra (PerkinElmer).

2.7. Electrophoretic mobility shift analysis

Electrophoretic mobility shift analysis was carried out using LightShift Chemiluminescent EMSA kit (Thermo Fisher Scientific) and nuclear extracts from HNF4α-transfected cells. The following double-stranded probes were used (mutations are indicated as bold and underlined); the HNF4α binding site at -13/-17 in the human megalin promoter (wild-type (WT); 5′-ctccgcgtctaagctagccgaggggg-3′ and 5′-cggccctgcactttgcagcgcggg-3′, mutant (Mut); 5′-ctccgcgtctagggtcggggcggg-3′ and 5′-cggccctgcacacctcagcgcggg-3′) and the HNF4α binding site at -203/-192 in the mouse ornithine transcarbamylase (Otc) promoter as a positive control (5′-gtctggctaatcgaagggccaggagacgacgagggggaaggg-3′ and 5′-cctgggtgtattgtaaattgtaggacgagggggaaggg-3′) [7]. Nuclear extracts (3 μg) and the 5′-biotin labeled probe of the megalin promoter (WT) were added and the reaction mixture incubated on ice for 10 min. For competition experiments, a 50-fold excess of the un-labeled megalin (Mut) or Otc probes was added to the reaction mixture and the mixture was incubated on ice for 10 min prior to the addition of the 5′-biotin labeled probe. For supershift analysis, 1 μg of anti-HNF4α or anti-PPARβ antibodies (Santa Cruz Biotechnology) was added to the reaction mixture, and the mixture was incubated on ice for 10 min after the addition of the 5′-biotin labeled probe. DNA-protein complexes were fractionated by 7% PAGE, and blotted onto a Hybond-N+ membrane (GE healthcare). After washing, DNA-protein complexes were visualized using detection module in the kit.

2.8. Chromatin immunoprecipitation

HNF4α-transfected HEK293T cells were fixed in 1% formaldehyde for 10 min at room temperature and chromatin immunoprecipitation was carried out using SimpleChIP Plus Enzymatic IP kit (Cell Signaling Technology) and anti-HNF4α antibody (Santa Cruz Biotechnology). Purified DNA was amplified by real-time PCR using ΔΔCt method. Enrichment of the HNF4α binding site was normalized to the input samples and expressed as fold-enrichment as compared to the control normal IgG antibody. Nucleotide sequences of the primers are as follows: megalin gene with HNF4α binding site (5′-gggtcggctaatcgaagggccaggagacgagggggaaggg-3′ and 5′-gtctggctaatcgaagggccaggagacgagggggaaggg-3′), megalin gene without HNF4α binding site (5′-gggtcggctaatcgaagggccaggagacgagggggaaggg-3′ and 5′-gtctggctaatcgaagggccaggagacgagggggaaggg-3′), OTC gene with HNF4α binding site (5′-atagggcggccggggaagggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggc
3. Results

3.1. Induced expression of proximal tubule-enriched genes by HNF4α

HNF4α upregulates many genes in liver, pancreas, small intestine, and colon [3,6-10,12]. PTECs also highly express HNF4α, but involvement in PTEC function by HNF4α has not been investigated in detail. Thus, we analyzed to identify novel HNF4α target genes that are highly expressed in PTECs. By overexpression of HNF4α in human PTEC-derived HK-2 cells, we investigated whether HNF4α has a potential to induce the expression of proximal tubule-enriched genes. When HNF4α expression vector was transfected into HK-2 cells, HNF4α mRNA and protein was strongly induced (Fig. 1A and B). Then, we compared expression of 82 genes including transporters, ion channels, and receptors that are highly expressed in the proximal tubules. Of these, expression of 78 genes was induced 0.5–1.5-fold, or no expression by HNF4α (Supplementary Table 2). Expression of 4 genes including SLC4A1, 7A7, 16A4, and megalin was increased more than 1.5-fold by HNF4α (Fig. 1C). Similar results were observed in HEK293T cells (Fig. 1D-F). In rat kidney, HNF4α was shown to bind to Slc22a1, Slc22a6, and Slc22a8 promoters [20], but the expression of these genes was not induced by HNF4α, or no expression in HK-2 cells (Supplementary Table 2). Of these, a noteworthy result was that expression of megalin was markedly induced more than 5- and 15-fold by HNF4α in HK-2 and HEK293T cells, respectively (Fig. 1C and F). Megalin plays an important role in reabsorption of albumin and low molecular weight proteins by endocytosis [23]. Interestingly, because megalin is involved in obesity/metabolic syndrome-related CKD [16], we investigated whether HNF4α directly transactivates the expression of megalin.

3.2. Transactivation of megalin promoter by HNF4α

By the JASPAR, an open access database for transcription factor binding sites, five HNF4α binding sites with high score were expected in the human megalin promoter region from -4000 to +200 from the transcription start site. Thus, promoter analysis was performed to determine whether HNF4α can transactivate the megalin promoter (Fig. 2A). The mouse ornithine carbamylase (Otc) gene containing functional two HNF4α binding sites was strongly transcativated by HNF4α [7]. The megalin promoters of -3996/+200 fragment containing five HNF4α binding sites and -3135 and -2071/+200 fragments containing two HNF4α binding sites at -1525/-1511 and -6/+9 were transactivated by approximately 15–20 fold by HNF4α (Fig. 2A). The -806/+200 fragment containing only one HNF4α binding site at -6/+9 was still transactivated by HNF4α, but the promoter activity was decreased by approximately 5-fold, indicating that two regions at -1525/-1511 and -6/+9 might be essential for transactivation of the megalin gene by HNF4α. Thus, mutations were introduced into these HNF4α binding sites, resulting that the promoter activity of the mutant at -6/+9 (MT1) was decreased by approximately 80%, but the mutant at -1525/-1511 (MT2) had no significant difference in the promoter activity compared with wild-type promoter (Fig. 2B). These data indicate that the region at -6/+9 of the megalin promoter is a critical cis-element for transactivation by HNF4α.

3.3. Binding of HNF4α to the megalin promoter

The binding site at -6/+9 of the megalin gene was highly conserved among species (Fig. 3A). To confirm the direct binding of HNF4α at -6/+9 in the megalin promoter, electrophoresis mobility shift analysis was performed (Fig. 3B). Nuclear extracts from HNF4α-transfected
HEK293T bound to biotin-labeled probe including the -6/+9 fragment of the megalin promoter (lane 2, the lower open arrow). This complex band was eliminated by the addition of excesses of unlabeled probe containing an HNF4α binding site in the mouse Otc promoter (lane 3) and the unlabeled megalin probe containing the -6/+9 fragment (lane 4), but not the unlabeled megalin probe whose mutations were introduced into the HNF4α binding site at the -6/+9 region (lane 5). The complex was supershifted by anti-HNF4α antibody, not by anti-PPARγ antibody (lanes 6 and 7, the upper closed arrow), indicating that HNF4α directly binds to the megalin promoter. Furthermore, chromatin immunoprecipitation analysis using HNF4α-transfected HEK293T cells showed that HNF4α indeed bound to the HNF4α binding sites of the OTC promoter as the positive control (Fig. 3C). HNF4α strongly bound to the megalin promoter region, suggesting that HNF4α directly and physiologically binds to the promoter region of the megalin gene.

4. Discussion

HNF4α is a master regulator in liver, and many function and the target genes of HNF4α have been identified [7–9]. Similarly, function of HNF4α in pancreatic β-cells and intestinal epithelial cells was investigated [10,12]. HNF4α also highly expresses in PTECs in kidney, but function of HNF4α in the PTECs has been poorly investigated due to lack of proximal tubule-specific Hnf4a-null mice. Thus, we aimed to identify novel HNF4α target genes that are highly expressed in the PTECs by HNF4α overexpression system.

Expression of 3 SLC transporters for chloride/bicarbonate (SLC4A1) [24], cationic amino acids/neutral amino acids (SLC7A7) [25], and orphan monocarboxylate (SLC16A4) [26], was upregulated more than 1.5-fold by HNF4α in human PTEC-derived HK-2 cells, these upregulated SLC transporters may be the novel target genes for HNF4α in the PTECs. HNF4α binds to Slc22a1, Slc22a6, and Slc22a8 promoters in rat kidney [20], but the expression of these genes was not induced by HNF4α, or no expression in HK-2 cells. Thus, HNF4α only binds to these promoters, and transactivation of these genes may not be induced in human PTECs.

Interestingly, expression of megalin was strongly induced by HNF4α. Megalin was first discovered as the pathological antigen of Heymann nephritis in the rat proximal tubule and cloned as gp330/megalin that is a large glycoprotein belonging to LDL receptor superfamily [27,28]. Subsequently, megalin was found to be a receptor for a large number of ligands such as calcium ion, retinol-binding proteins, vitamin D-binding proteins, albumin, transthyretin, and liver-type fatty acid binding protein [29]. Whole body megalin-null mice indeed result in low molecular weight proteinuria of serum carrier proteins such as retinol-binding proteins and vitamin D-binding proteins, and the similar symptoms occur in Fanconi syndrome [30]. Kidney-specific megalin-deficient mice also exhibit decreased plasma vitamin D, probably due to decreased uptake of vitamin D-binding protein/25-OH vitamin D3 complex, and result in hypocalcemia and severe osteopathy, suggesting that megalin is a critical receptor for calcium homeostasis and bone metabolism [31]. Mutations in megalin gene were found in families with Donnai-Barrow syndrome and facio-oculo-acoustico-renal syndrome, revealing that megalin is an important regulator of many compounds in the bloodstream [32]. Interestingly, PTECs in high-fat diet fed wild-type mice, but not kidney-specific megalin-null mice, showed autolysosomal dysfunction with autophagy impairment, indicating that megalin might be associated with CKD development and progression [33].

Although transcriptional regulation of megalin have not been investigated in detail, it was reported that peroxisome proliferator-activated receptor α (PPARα) and γ (PPARγ) positively regulates the
expression of megalin through the binding sites in the promoter region [34]. However, expression of megalin was only about 2-fold induced by the ligands for PPARα, indicating that other transcription factors synergistically regulate expression of megalin gene with PPARα/γ. Because HNF4α induced megalin expression about 15-fold, HNF4α may be an important factor to transactivate the megalin gene in PTECs. Several studies have reported that HNF4α and PPARα could share the binding sites in the promoter regions [35]. For example, HNF4α and PPARα bind to the same sequence in acyl-CoA thioesterase 1 (Acot1) promoter [36]. In the report, expression of Acot1 was upregulated by treatment of ligand for PPARα, but the expression of Acot1 is repressed in the presence of HNF4α. Conversely, PPARα was shown to bind to multiple binding sites in the megalin promoter [34], but no transactivation by HNF4α was observed through these binding sites in this study. HNF4α bound to the other region including the transcription start site and transactivated the expression of megalin, indicating that HNF4α and PPARα may positively regulate the expression of megalin though different binding sites in PTECs. Because saturated and unsaturated free fatty acids and their derivatives are endogenous ligands for PPARα [37] and megalin uptakes albumin-bound fatty acids into PTECs, PPARα might be transactivated by these fatty acids uptaken through megalin. Because of expression of PPARα is positively regulated by HNF4α [38], HNF4α may play a dual role in megalin expression through direct regulation of megalin itself and indirect regulation of megalin by PPARα.

In conclusion, HNF4α was found to upregulate many genes including transporters and megalin that are highly expressed in PTECs. Of these, HNF4α strongly upregulated megalin expression through an HNF4α binding site in the promoter region. Because dysfunction of transporters and megalin causes many diseases including CKD, HNF4α...
may play a critical role in maintaining the function of PTECs.

Funding

This work was supported in part by grants from the Ministry of Education, Culture, Sports, Science, and Technology of Japan (Grant-in-Aid for Scientific Research, No. 25460490).

Conflict of interest

The authors declare no conflicts of interest associated with this manuscript.

Appendix A. Transparency document

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.bbrep.2018.11.010.

Appendix B. Supplementary material

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.bbrep.2018.11.010.

References

[1] W. Zhong, J. Mirkovitch, J.E. Darnell Jr., Tissue-specific regulation of mouse hepatocyte nuclear factor 4 expression, Mol. Cell Biol. 14 (1994) 7276–7284.

[2] T. Drewes, S. Senkel, B. Holewa, G.U. Ryter, Human hepatocyte nuclear factor 4 isoforms are encoded by distinct and differentially expressed genes, Mol. Cell Biol. 16 (1996) 925–931.

[3] T.O. Odom, N. Zhang, D.B. Gordon, G.W. Bell, N.J. Rinaldi, H.L. Murray, T.L. Volkert, J. Schreiber, P.A. Bolfe, D.K. Gifford, E. Frankel, G.U. Ryter, A. Young, Control of pancreas and liver gene expression by HNF transcription factors, Science 303 (2004) 1378–1381.

[4] W.S. Chen, K. Manova, D.C. Weinstein, S.A. Duncan, A.S. Plump, V.R. Prezioso, T.L. Volkert, J. Schreiber, P.A. Rolfe, D.K. Gifford, Control of pancreas and liver gene expression by HNF transcription factors, Science 303 (2004) 1378–1381.

[5] G.P. Hayhurst, Y.H. Lee, G. Lambert, J.M. Ward, F.J. Gonzalez, Hepatocyte nuclear factor 4alpha (nuclear receptor 2A1) is essential for maintenance of hepatic gene expression and lipid homeostasis, Mol. Cell Biol. 21 (2001) 1393–1403.

[6] Y. Inoue, G.P. Hayhurst, J. Inoue, M. Mori, F.J. Gonzalez, Defective ureagenesis in mice carrying a liver-specific hepatocyte nuclear factor 4alpha (HNF4alpha) knockout, J. Biol. Chem. 277 (2002) 25257–25265.

[7] S. Matsuo, M. Ogawa, M.U. Muckenthaler, Y. Mizui, S. Sasaki, T. Fujimura, R.L. Chevalier, The proximal tubule is the primary target of injury and progression of kidney disease: role of the glomerulotubular junction, Am. J. Physiol. Ren. Physiol. 311 (2016) F145–F161.

[8] I. Ogg, G. Campanile, B. Bijel, C. Wang, V.S. Sabisbiets, T. Ichimura, B.D. Humphreys, J.V. Bonventre, Targeted proximal tubule injury triggers interstitial fibrosis and glomerulosclerosis, Kidney Int. 82 (2012) 172–183.

[9] V. Vallon, K.A. Platt, R. Curand, J. Schroth, J. Whaley, S.C. Thomson, H. Koeppel, T. Rieg, SORT7 mediates glomerular reabsorption in the early proximal tubule, J. Am. Soc. Nephrol. 22 (2011) 104–112.

[10] A. Miura, K. Yamagata, M. Kakei, H. Haga, K. Sharma, M. Yanagita, Severity and frequency of proximal tubule injury determines renal prognosis, J. Am. Soc. Nephrol. 27 (2016) 2393–2406.

[11] R.L. Chevalier, The proximal tubule is the primary target of injury and progression of kidney disease: role of the glomerulotubular junction, Am. J. Physiol. Ren. Physiol. 311 (2016) F145–F161.

[12] T. Kanazawa, A. Konno, Y. Hashimoto, Y. Kon, Enterocyte-specific regulation of the apical nutrient transporter SLC3A21 (EQT1) by transcriptional and epigenetic networks, J. Biol. Chem. 288 (2013) 33813–33823.

[13] T.P. Gallegos, G. Martovetsky, V. Kouznetsova, K.T. Bush, S.K. Nigam, Organic anion and cation SLC22A ‘drug’ transporter (Oct1, Oct3, and Oct1) regulation during development and maturation of the kidney proximal tubule, PLoS One 7 (2012) e40796.

[14] M.J. Ryan, G. Johnson, K. Sikk, S.M. Fuestenberg, R.A. Zager, B. Torok-Storb, HK-2: an immortalized proximal tubule epithelial cell line from normal adult human kidney, Kidney Int. 45 (1994) 48–57.

[15] S. Signorini, M. Stoian, F. Parviz, C. Matullo, W.D. Garrison, L. Savatski, J.W. Adamson, G. Ning, D. Fotiadis, Y. Kanai, M. Palacin, The SLC3 and SLC7 families of amino acid transporters, Mol. Asp. Med. 34 (2013) 557–566.

[16] A. Saito, S. Noto, I. Takeda, Molecular mechanisms of receptor-mediated endocytosis in the renal proximal tubular epithelium, J. Biol. Chem. 310 (2010) 40327–40338.

[17] S.I. Alper, Molecular physiology and genetics of Na–independent SLC4 anion exchangers, J. Exp. Biol. 212 (2009) 1672–1683.

[18] D. Fotiadis, Y. Kanai, M. Palacin, The SLC3 and SLC7 families of amino acid transporters, Mol. Asp. Med. 34 (2013) 139–158.

[19] A.P. Haelestrap, The SLC16 gene family - structure, regulation and role in health and disease, Mol. Asp. Med. 34 (2013) 337–349.

[20] D. Kerjaschki, M.G. Farquhar, The pathogenic antigen of Heymann nephritis is a membrane glycoprotein of the renal proximal tubule brush border, Proc. Natl. Acad. Sci. USA 79 (1982) 5557–5561.

[21] A. Saito, S. Pietromonaco, A.K. Loo, M.G. Farquhar, Complete cloning and sequencing of rat grp330/‘megalin,’ a distinctive member of the low density lipoprotein receptor gene family, Proc. Natl. Acad. Sci. USA 91 (1994) 9725–9729.

[22] E.I. Christensen, P.J. Verroust, R. Nielsen, Receptor-mediated endocytosis in renal proximal tubule, Pflug. Arch. 458 (2009) 1039–1048.

[23] J.K. Lehste, B. Rolinski, H. Vorum, J. Hilpert, A. Nykjaer, C. Jacobsen, P. Auscoturier, J.O. Moskau, A. Otto, E.I. Christensen, T.E. WillNow, Megalin knockout mice as an animal model of low molecular weight proteinuria, Am. J. Pathol. 155 (1999) 1361–1370.

[24] J.K. Lehste, F. Melsen, F. Wellner, J. Schlichting, I. Renner-Muller, T.F. Andreason, E. Wolf, S. Bachmann, A. Nykjaer, T.E. WillNow, Hypocalcemia and osteopathy in mice with kidney-specific megalin gene defect, FASEB J. 17 (2003) 247–249.

[25] S. Cantarini, L. Al-Gazali, R.S. Hill, D. Donnai, G. Black, E. Bieth, N. Chassagne, D. Komeyer, K. Tebbi, M. Lescoatres, G. Chen, T. Liu, D.T. MacLaughlin, K.M. Noonan, M.K. Russell, C.A. Walsh, P.K. Donahoe, B.R. Pober, Mutations in LRP2, which encodes the multiligand receptor megalin, and -gamma: implications for PPARs’ roles in renal function, PLoS One 6 (2011) e29673.

[26] S. Kuswahara, M. Hosojima, K. Aoki, D. Nakano, T. Saigawa, H. Kakasawa, R. Kaseda, Y. Yasukawa, T. Ishikawa, A. Suzuki, H. Hato, S. Kageyama, T. Tanaka, N. Kitamura, I. Narita, M. Komatsu, A. Nishiyama, A. Saito, Megalin-mediated tubuloglomerular alterations in high-fat diet-induced kidney disease, Am. J. Physiol. Ren. Physiol. 311 (2016) F145–F161.

[27] F. Cabezás, J. Lagos, C. Cedes, P.C. Cio, M. Bronfman, M.P. Marzolo, Megalin/ LRPI expression is induced by peroxisome proliferator-activated receptor -alpha and -gamma: implications for PPARs’ roles in renal function, PLoS One 6 (2011) e16794.

[28] J. Chamouton, N. Latruffe, PPARalpha/HNF4alpha interplay on diverse responsiveness of PPARs’ roles in renal function, PLoS One 6 (2011) e16794.

[29] C. Sasaki et al. Biochemistry and Biophysics Reports 17 (2019) 87–92