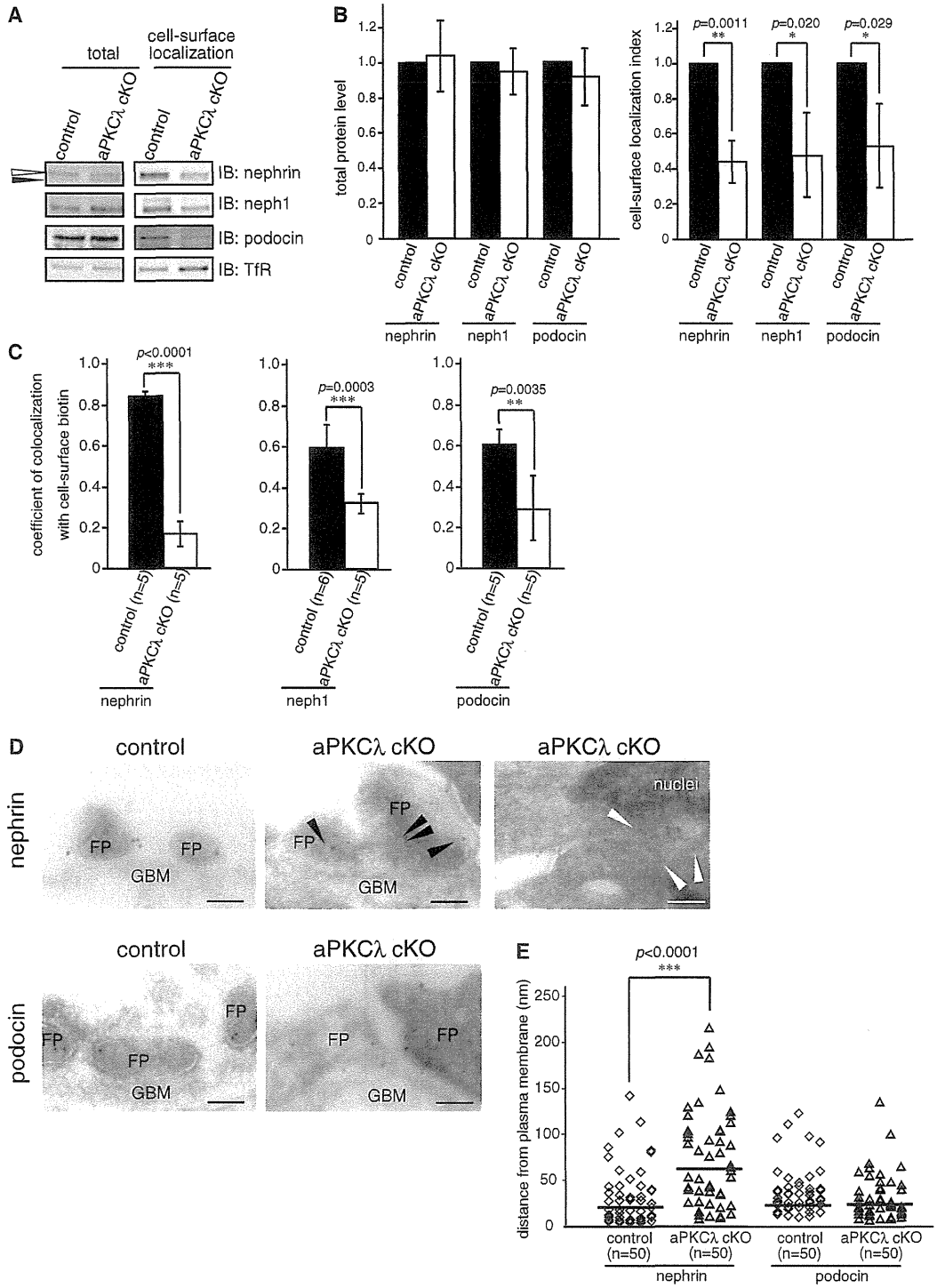

Fig. 5 aPKC is required for the cell-surface localization of SD components *in vivo*. (**A**) aPKC cKO and control mice at P10 or P11 were transcardially perfused with 2 mg/ml sulfo-NHS-SS-biotin/PBSCM for 5 min. Then, the kidneys were lysed and biotinylated proteins isolated with streptavidin sepharose and detected by immunoblot. The white arrowhead represents the mature-glycosylated, cell-surface form, and the black arrowhead represents the *N*-glycosylated, ER-form of nephrin. (**B**) Quantification of the results in (A). The values were normalized to control mice and are the mean ± SD of three independent experiments. The *P* values were determined by two-tailed Student's *t*-test. (**C**) The cell-surface biotinylated aPKC cKO and control kidney were immunostained with nephrin, neph1 or podocin and biotin (Supplementary Fig. S5), and the colocalization coefficient was calculated with LAS-AF software provided by Leica. The *P* values were determined by two-tailed Student's *t*-test. (**D**) The ultrathin cryosections of aPKC cKO and control kidney were labelled with anti-nephrin or anti-podocin antibodies followed by 10 nm gold particle-conjugated secondary antibody. Black arrowheads represent nephrin localized to the intracellular region, and white arrowheads represent nephrin localized to the rough ER. FP, foot process; GBM, glomerular basement membrane. (**E**) The distance of gold particle-labelled nephrin or podocin from the plasma membrane in aPKC cKO podocytes was compared with that of the control kidney. The *P* values were determined by two-tailed Mann–Whitney *U*-test.

the parallel experiments on nephrin-expressing cultured epithelial cells with direct evaluation of exocytosis of newly synthesized nephrin. Finally, the involvement of aPKCλ in the exocytosis of nephrin was confirmed *in vivo* using aPKCλ cKO mice.

Reduced expression and abnormal distribution of SD components are observed in several nephrotic syndromes in humans, including minimal change nephrotic syndrome, focal and segmental glomerulosclerosis, lupus nephritis, diabetic nephropathy and membranous nephropathy (*8–12*) and also in various disease models (*15–18*). However, little is known about the trafficking of SD components in the intact SD (*52*, *53*). Our present findings indicating the importance of the rapid turnover of SD components to the cell surface are consistent with these previous observations, highlighting the importance of endocytosis of SD components in the maintenance of SD integrity. Taken together with previous observations, our present results suggest that the balance between exocytosis and endocytosis is tightly associated not only with the maintenance of SD integrity, but also with the pathogenesis of glomerular diseases. To elucidate the alteration of the high turnover rate of cell-surface SD components under disease conditions would provide a new pathophysiologic basis of proteinuria in glomerular diseases.

What is the physiological reason for rapid turnover of cell-surface SD components? It is suggested that this model can explain how podocytes replace clogged SD components with new ones to prevent overall SD clogging and minimize the unnecessary breakdown of SD structure (*50*). In addition, the high turnover of cell-surface SD components further enables the remodelling of SD architecture in response to various physiological parameters such as blood flow-dependent change of glomerular capillary diameter (*22*).

The molecular mechanisms regulating the turnover of SD components at the cell surface: the role of aPKC

Although we and other groups have shown that aPKC plays an essential role in the maintenance of SD integrity (*30*, *31*), the precise role of aPKC has been largely unknown. In this study, we demonstrated that aPKC plays a critical role in the cell-surface localization of SD components, including nephrin, possibly through the exocytosis of newly synthesized ones. Furthermore, our results suggest that Par3, a component of the aPKC-Par complex, is also required for the cell-surface localization of proteins including nephrin. As previously demonstrated in columnar epithelial cells, aPKC forms a complex with Par3 in podocytes (*30*). Therefore, these observations suggest that aPKC and Par3 might jointly regulate the cell-surface localization of SD components.

Previous studies suggested that podocin is necessary for the cell-surface localization of nephrin *in vitro* (*6*, *7*). However, we show that nephrin can be targeted to the plasma membrane without podocin since HCT116-nephrin cells do not express it. Moreover, aPKC is required for the cell-surface localization of both nephrin and neph1 in kidneys and isolated glomeruli. These observations suggest that aPKC can regulate the cell-surface localization of nephrin independently of podocin. The molecular mechanisms regulating the exocytosis of nephrin through aPKC are currently uncertain. One possible mechanism is that aPKC regulates the exocytosis of nephrin through the modulation of the exocyst complex, the octameric complex that tethers transport vesicle to the plasma membrane (*54*). Since several studies have suggested that aPKC associates with the exocyst complex (*35*, *55*), the kinase activity of aPKC might regulate the formation or appropriate localization of the exocyst complex for the exocytosis of nephrin. In podocytes, it is suggest that aPKC form a complex with Par3 and nephrin at the plasma membrane (*30*). Furthermore, it has been reported that combined deletion of aPKCλ and aPKCζ isoforms in podocytes associates with incorrectly positioned Golgi apparatus (*56*). Based on these observations and our results, aPKC might regulate multiple steps in the exocytosis of nephrin; transport from ER to Golgi apparatus and from Golgi apparatus to plasma membrane.

The activation of aPKC is regulated by multiple mechanisms including the phosphatidylinositol 3-kinase (PI3K)-dependent pathway (*57*), and aPKC under control of PI3K has been implicated in insulin-stimulated surface mobilization of GLUT4 (*44*). Because nephrin interacts with PI3K in a tyrosine phosphorylation-dependent manner and activates PI3K signalling (*58*), it is plausible that aPKC is activated downstream of nephrin through PI3K. In addition, it has been demonstrated that the tyrosine phosphorylation of nephrin by Src family kinases triggers endocytosis of nephrin (*19*). Based on these previous observations and our current findings, we propose a model of negative feedback mechanisms to maintain cell-surface localization of nephrin. First, cell-surface nephrin that is tyrosine-phosphorylated by Src family kinases leads to the binding and activation of PI3K and endocytosis. In turn, activated PI3K leads to the activation of downstream effector aPKC, which leads to the exocytosis of newly synthesized nephrin to cell surface.

The correlation between the alteration of the kinase activity of aPKC and the pathological conditions present in glomerular diseases is still unclear. It has been suggested that the kinase activity of aPKC is regulated by both insulin and epidermal growth factor through PI3K (*57*, *59*), and that these observations imply that the change in the composition of serum circulating factors may alter the kinase activity of aPKC in pathological conditions. To investigate the expression, localization or kinase activity of aPKC and the upstream factors in the pathogenesis or recovery phase of proteinuria will provide novel insights into the pathophysiological basis, and a potential therapeutic target, for glomerular diseases.

Taken together, we present a novel model for the maintenance of SD integrity, where the cell-surface localization of SD components is dynamically regulated by persistent and rapid turnover of these proteins at the cell surface. Furthermore, the cell polarity regulator aPKC plays a critical role in the cell-surface localization of SD components through the regulation of

exocytosis. As mentioned above, the disturbance in the cell-surface localization of SD components is tightly associated with the pathogenesis and progression of proteinuria. Therefore, the signalling leading to the inhibition of aPKC activity would be one of the therapeutic targets for proteinuria. These results provide a new insight into the pathophysiological basis for glomerular diseases and shed light on the exocytosis pathway as a potential therapeutic target for proteinuria.

Supplementary Data

Supplementary Data are available at *JB* Online.

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Conflict of Interest

None declared.

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V.　資料

爪膝蓋骨症候群 (ネイルパテラ症候群) および *LMX1B* 関連腎症

○ 概要

1, 概要

爪膝蓋骨症候群 (ネイルパテラ症候群) は爪形成不全、膝蓋骨の低形成あるいは無形成、腸骨の角状突起 (iliac horn)、肘関節の異形成を 4 主徴とする遺伝性疾患である。しばしば腎症を発症し、一部は末期腎不全に進行する。原因は *LMX1B* 遺伝子変異である。

爪、膝蓋骨、腸骨などの変化を伴わず、腎症だけを呈する nail-patella-like renal disease (NPLRD) や巣状分節性糸球体硬化症患者にも *LMX1B* 遺伝子変異を原因とする例が存在する。これら一連の疾患群は *LMX1B* 関連腎症と呼ばれる。

2, 原因

爪膝蓋骨症候群の原因は *LMX1B* の変異である。これまでに 130 種類以上の変異が同定されている。NPLRD の一部、また巣状分節性糸球体硬化症患者やステロイド抵抗性ネフローゼ症候群患者の一部からも *LMX1B* 変異が見いだされている。

病態発症メカニズムとしては *LMX1B* 変異による糸球体上皮細胞機能障害が推定される。

3、症状

(1) 爪膝蓋骨症候群 (ネイルパテラ症候群)

爪形成不全、膝蓋骨の低形成あるいは無形成、腸骨の角状突起 (iliac horn)、肘関節の異形成がみられるが、このうちの一つあるいは複数の症状のみを呈する場合が有る。約半数に腎症を合併する。症状としては無症候性の蛋白尿や血尿がみられる。特に高度の蛋白尿によりネフローゼ症候群を呈することがある。15%の症例で腎機能が進行性に悪化し末期腎不全に至る。

組織学的には光学顕微鏡レベルでは特異的な所見はないが、特徴的な所見としては電子顕微鏡所見では糸球体基底膜が不規則に肥厚し、またその緻密層に虫食い像 (moth-eaten appearance) や III 型コラーゲンの沈着を認める。

(2) *LMX1B* 関連腎症

腎外合併症はなく、腎症 (蛋白尿、血尿)、腎機能障害、腎不全を呈する。

爪膝蓋骨症候群の腎組織像と同様の電子顕微鏡所見を示す場合と、示さない場合が

報告されている。

4、治療法

爪膝蓋骨症候群における爪、膝、肘関節の異常に対しては効果的な治療法はない。

腎症に対しても特異的な治療は存在しない。しかし近年アンギオテンシン変換酵素阻害薬やアンギオテンシンII受容体拮抗薬などの腎不全予防治療が一定の効果を呈することが知られている。腎不全に至った場合には維持透析あるいは腎移植を要する。

5、予後

腎症が生命予後を規定する。3-5割に腎症を合併する。小児期に発症することも多い。そのうち1-3割で末期腎不全へと進行する。

○ 要件の判定に必要な事項

1. 患者数
   総患者数約500人程度と推計される
2. 発病の機構
   *LMX1B*遺伝子異常による
3. 効果的な治療方法
   未確立(対症療法のみである)
4. 長期の療養
   必要(腎不全に対する治療や腎代替療法が必要となる場合が有る)
5. 診断基準
   あり(日本腎臓学会と研究班が共同で作成した基準有り)
6. 重症度分類
   慢性腎臓病重症度分類で重症に該当するもの、あるいはいずれの腎機能であっても尿蛋白/クレアチニン比　0.5g/g・Cr以上のものを、重症として扱う。

○ 情報提供元

「*LMX1B*関連腎症の実態調査および診断基準の確立」研究班
難治性疾患等政策研究事業（難治性疾患政策研究事業）(H26-27)
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〈診断基準-爪膝蓋骨症候群〉

主項目を満たし、かつ副項目１項目以上を有し、さらに鑑別疾患を除外したものを爪膝蓋骨症候群と診断する。

主項目

爪の低形成あるいは異形成

（手指に多く、特に母指側に強い。程度は完全欠損から低形成まで様々である。三角状の爪半月のみを呈する場合もあり、軽症であると気づかれにくい。）

副項目

1. 膝蓋骨形成不全
2. 肘関節異常
3. 腸骨の角状突起
4. *LMX1B* 遺伝子のヘテロ接合体変異

参考項目

1. 爪膝蓋骨症候群の家族歴
2. 腎障害　（血尿、蛋白尿、あるいは腎機能障害）
3. 腎糸球体基底膜の特徴的電顕所見

   (腎障害が有った場合に腎生検を検討するが、本症の診断上は必須ではない。病理像としては腎糸球体基底膜の肥厚と虫食い像”moth-eaten appearance”が特徴的である。肥厚した糸球体基底膜中央の緻密層やメサンギウム基質内に III 型コラーゲン繊維の沈着が見られる。これらの線維成分はリンタングステン酸染色あるいはタンニン酸染色で染色される)

鑑別診断

1. Meier-Gorlin 症候群 (OMIM224690),
2. Genitopatellar 症候群 (OMIM606170)
3. DOOR 症候群 (OMIM220500)
4. ８トリソミーモザイク症候群
5. Coffin-Siris 症候群 (OMIM135900) / BOD 症候群 (OMIM113477)
6. RAPADILINO 症候群 (OMIM266280)

〈診断基準-*LMX1B*関連腎症〉

主項目の三つを満たし、副項目の少なくとも一つを満たすものを *LMX1B* 関連腎症と診断する。

主項目

1. 腎障害　（血尿、蛋白尿、あるいは腎機能障害）
2. 爪膝蓋骨症候群の診断基準を満たさない
3. 腎障害を来す他の原因（遺伝子異常など）を有さない

副項目

1. *LMX1B* 遺伝子のヘテロ接合体変異
2. 腎糸球体基底膜の特徴的電顕所見

（腎生検病理において、腎糸球体基底膜の肥厚と虫食い像”moth-eaten appearance”を認め、さらにリンタングステン酸染色あるいはタンニン酸染色により基底膜内に線維成分が染色される）

注.　尿所見異常あるいは腎機能障害あり、腎生検所見で腎糸球体基底膜の特徴的電顕所見が有った場合あるいは常染色体優性遺伝形式を示す家族歴を有する場合に *LMX1B* 遺伝子検査を考慮する。

〈重症度分類　（爪膝蓋骨症候群および *LMX1B* 関連腎症共通）〉

慢性腎臓病重症度分類で重症に該当するもの（下図赤）、あるいはいずれの腎機能であっても尿蛋白／クレアチニン比　0.5g/g・Cr 以上のものを、重症として扱う。

原疾患		蛋白尿区分		A1	A2	A3
糖尿病		尿アルブミン定量（mg/日） 尿アルブミン/Cr 比（mg/gCr）		正常	微量アルブミン尿	顕性アルブミン尿
				30 未満	30～299	300 以上
高血圧 腎炎 多発性嚢胞腎 移植腎 不明 その他		尿蛋白定量（g/日） 尿蛋白/Cr 比（g/gCr）		正常	軽度蛋白尿	高度蛋白尿
				0.15 未満	0.15～0.49	0.50 以上
GFR区分（mL/分/1.73 m²）	G1	正常または高値	≧90			
	G2	正常または軽度低下	60～89			
	G3a	軽度～中等度低下	45～59			
	G3b	中等度～高度低下	30～44			
	G4	高度低下	15～29			
	G5	末期腎不全（ESKD）	＜15			

重症度は原疾患・GFR 区分・蛋白尿区分を合わせたステージにより評価する．CKD の重症度は死亡，末期腎不全，心血管死亡発症のリスクを緑 のステージを基準に，黄 ，オレンジ ，赤 の順にステージが上昇するほどリスクは上昇する．（KDIGO CKD guideline 2012 を日本人用に改変）

※なお、症状の程度が上記の重症度分類等で一定以上に該当しないが、高額な医療を継続することが必要な者については、医療費助成の対象とする。

膠原線維糸球体沈着症：Collagenofibrotic Glomerulopathy

○ 概要

1, 概要

糸球体沈着症のうち、非アミロイド、非免疫グロブリン由来の細線維の沈着による疾患群の一つである。光顕組織像では糸球体メサンギウム基質領域の拡大、糸球体内皮細胞下腔の開大がみられ、これらの部位に免疫染色で III 型コラーゲンの沈着を認める。メサンギウム細胞の増加は顕著ではない。電子顕微鏡で観察すると、メサンギウム領域と糸球体内皮細胞下腔に可視型 III 型コラーゲンの沈着が観察される。

2, 原因

ほとんどの症例で血中の III 型プロコラーゲンのN端ペプチド量が増加していることから、III 型コラーゲンの産生増加が原因の一つと考えられている。血中ヒアルロン酸の増加も報告されており、膠原線維とプロテオグリカンの産生異常が背景にある可能性がある。家族性の発症例も報告されているが、遺伝的な原因については不明である。

(注意) III 型プロコラーゲンとヒアルロン酸の測定は肝疾患が保険適用である。

3、症状

小児から成人にかけて、すべての年齢で発症する。多くの症例で蛋白尿や浮腫を認める。6 割程度の患者でネフローゼ症候群レベルの蛋白尿を呈する。血尿は比較的軽度である。腎機能低下例では高血圧と貧血を高率に認める。末期腎不全に進行する症例も存在する。

腎外症状は認めない。

4、治療法

高血圧や浮腫に対する支持的治療が行われるが、現時点で特異的な治療法はない。

腎不全に至った症例は腎代替療法が必要となる。

5、予後

2～6 割が末期腎不全へと進行する。

○ 要件の判定に必要な事項

1. 患者数

   100 人未満

2. 発病の機構

   不明（III 型コラーゲンとヒアルロン酸産生増加が推定されている）

3. 効果的な治療方法

   未確立 (対症療法のみである)

4. 長期の療養

   必要 (腎不全に対する治療や腎代替療法が必要となる場合が有る)

5. 診断基準

   なし

6. 重症度分類

   慢性腎臓病重症度分類で重症に該当するもの、あるいはいずれの腎機能であっても尿蛋白／クレアチニン比　0.5g/g・Cr 以上のものを、重症として扱う。

○ 情報提供元

「*LMX1B* 関連腎症の実態調査および診断基準の確立」研究班

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研究代表者　東京大学医学部小児科　張田豊

〈重症度分類〉

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高血圧 腎炎 多発性嚢胞腎 移植腎 不明 その他		尿蛋白定量 (g/日) 尿蛋白/Cr 比 (g/gCr)		正常	軽度蛋白尿	高度蛋白尿
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	G3a	軽度～中等度低下	45～59			
	G3b	中等度～高度低下	30～44			
	G4	高度低下	15～29			
	G5	末期腎不全 (ESKD)	<15			

重症度は原疾患・GFR 区分・蛋白尿区分を合わせたステージにより評価する．CKD の重症度は死亡，末期腎不全，心血管死亡発症のリスクを緑 のステージを基準に，黄 ，オレンジ ，赤 の順にステージが上昇するほどリスクは上昇する．(KDIGO CKD guideline 2012 を日本人用に改変)

※なお、症状の程度が上記の重症度分類等で一定以上に該当しないが、高額な医療を継続することが必要な者については、医療費助成の対象とする。