アルポート症候群患者由来の変異を導入したCol4a5 R471Xマウスの性状解析とイルベサルタンの治療効果

Phenotype analysis and efficacy of irbesartan in Col4a5 R471X mice harboring a mutation similar to an Alport syndrome patient

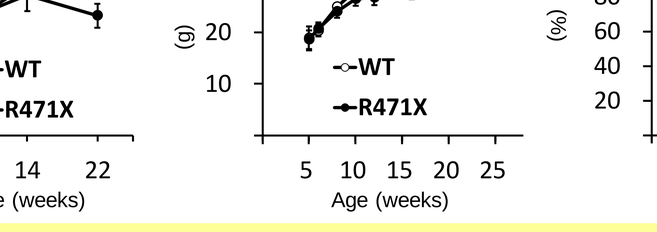
○階上 健太郎、松本 光晴、金子 まなみ、竹山 道康 （Axcelead Drug Discovery Partners株式会社・統合生物 ）

Purpose

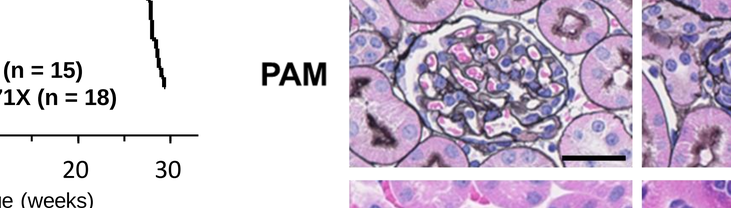
アルポート症候群（AS）は腎糸球体基底膜の異常に起因して若齢で慢性腎炎を呈し末期腎不全へ進行する遺伝性疾患である。ASの原因はIV型コラーゲンの遺伝子変異であり、患者の80%以上が**COL4A5**遺伝子に変異を持つX連鎖型アルポート症候群（XLAS）である。病因遺伝子は特定されているが、その進行機序は未解明であり、効果的な治療法も見つかっていない。そこで、XLAS患者由来のナンセンス変異を持つR471Xマウスを**CRISPR-Cas9**システムを用いて作製し、その性状解析を行った（Hashikami et al., *Biochem Biophys Rep.* 2019）。本マウスでは、尿中アルブミンが高値を示しており、腎臓の光顕、電顕所見からも、糸球体基底膜の異常が見られるなど、AS患者の臨床的特徴と一致する特徴を確認できた。今回さらに、**慢性腎不全**を呈する本モデルを用いてアンジオテンシンII受容体拮抗薬であるイルベサルタンの治療効果を検討したので報告する。

Methods

A.Targeting strategy for Col4a5 R471X mice



B. Protocol of Irbesartan treatment

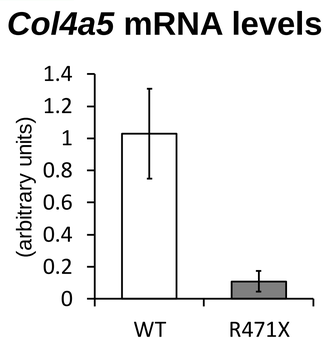


	Mouse	Treatment	Dose	N
1	WT	Vehicle	-	6
2	R471X	Vehicle	-	12
3	R471X	Irbesartan	50 mg/kg, p.o	12

WT: Wild type mice, R471X: Col4a5 R471X mice,
p.o.: oral administration
Methyl cellulose solution was used as a vehicle.

A) The *Col4a5* R471X mice were generated using the CRISPR-Cas9 system with ssODN which had a XLAS patient derived mutation. Their phenotypes were investigated by the measurement of urinary and blood biochemical parameters and light and electron microscopy to confirm whether hemizygous R471X male mice developed AS-like pathology. B) Therapeutic effect of irbesartan (50 mg / kg / day), an angiotensin II receptor antagonist, was examined in these mice. Repeated administration was performed for 12 weeks from 10 weeks of age, and urinary albumin was measured every 4 weeks. Data were shown as mean ± SD.

Results



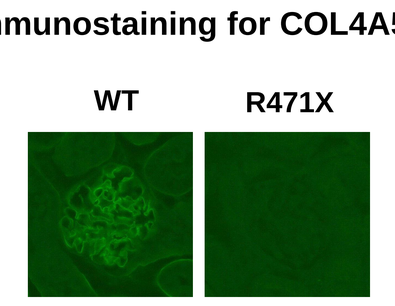


Fig 1: Confirmation of Col4a5 deletion.
The expression level of Col4a5 mRNA in the kidney was remarkably decreased in the R471X mice. The glomerular basement membrane was not stained in R471X mice, suggesting that COL4A5 was not expressed by nonsense mutation.

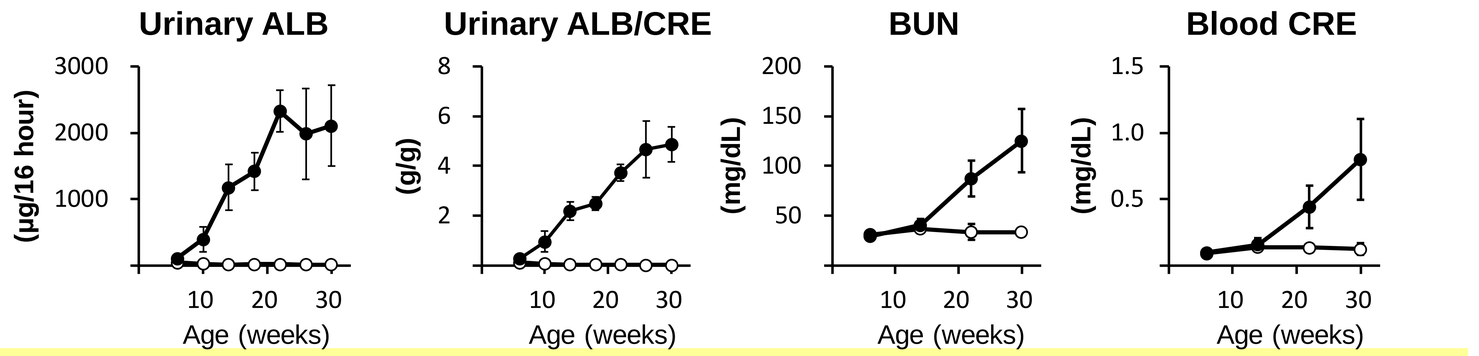


Fig 2: Urine and blood parameters.
Urinary albumin (ALB) levels showed an increasing tendency at 6 weeks of age in R471X mice, which increased with aging and remained high after 22 weeks of age (n=5–6). Urinary ALB corrected by creatinine (CRE), blood urea nitrogen (BUN), and blood CRE levels were also increased with aging.

Fig 3: Kidney weight, body weight, and survival curve.
Kidney and body weight normally increased in R471X mice until 14 weeks of age, but not increase after that. They started to die from 26 weeks of age and 72.2% of them died by 30 weeks of age.

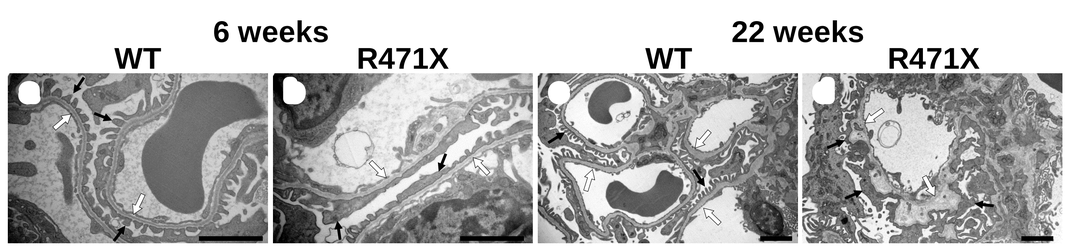


Fig 4: Transmission electron microscopy images of the glomerular basement membrane (GBM).
At 6 weeks of age, R471X mice showed focal thinning and mild irregularity in GBM, and focal foot process effacement in podocytes. At 22 weeks of age, marked irregularities such as thickening and lamination were showed in R471X mice. White and black arrows indicate GBM and podocyte foot processes, respectively. Scale bar: 2 μm.

Fig 5: Histological changes in kidney.
At 6 weeks of age, R471X mice revealed tuft collapse with crescentic formation and tubulointerstitial fibrosis (Scale bar: 50 μm). At 22 weeks of age, R471X mice showed glomerular collapse with extraglomerular hypercellularity (Scale bar: 200 μm). PAM; periodic acid silver-methenamine stain, HE; hematoxylin eosin stain, MT; Masson trichrome stain.

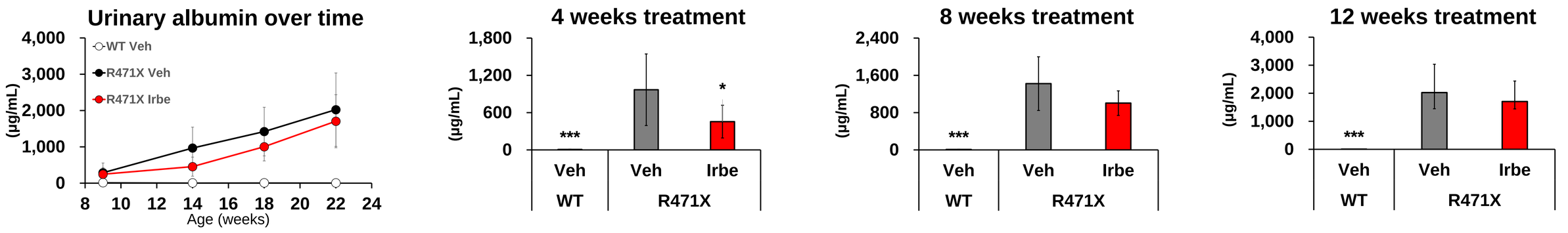


Fig 6: Effect of irbesartan on urinary albumin.
Urine was collected after 4, 8 and 10-week treatment at 14, 18 and 22 weeks of age, respectively. Irbesartan (50 mg/kg, p.o.) significantly inhibited elevation of urinary albumin at the time of 4 weeks treatment, but not at the time of 8- and 12-weeks treatment. *: p<0.05, ***: p<0.001 in R471X mice treated with vehicle vs.R471X mice treated with Irbesartan by Aspin-Welch t-test. Veh: vehicle, Irbe: irbesartan

Conclusion

CRISPR-Cas9システムを用いることで、短期間でAS患者由来の変異を持った*Col4a5* R471Xマウスを作製することができた。表現型解析の結果、本マウスはAS患者の臨床的特徴と一致する慢性腎不全様の病態を示すことが確認された。本マウスを用いた薬剤投与試験において、イルベサルタン群では、投与8、12週と病態が進行するにつれてその作用は減弱したものの、投与4週目ではベヒクル群と比較して尿中アルブミン上昇を有意に抑制した。本モデルマウスは、Alport症候群、および糸球体異常を有する慢性腎不全に対する創薬研究に非常に有用なツールであると考えられる。

COI開示: 開示すべきCOI関係にある企業などはありません。