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Major Vault Protein Contributes to Tubulo-interstitial Inflammation and Fibrosis in a Murine Model of Chronic Kidney Disease

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Introduction

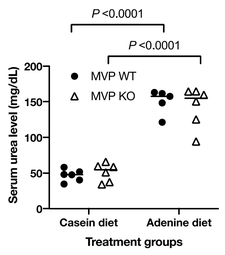
Chronic kidney disease (CKD) is a global health problem characterized by interstitial fibrosis and tubular atrophy, and progression to kidney failure. There is no effective treatment for kidney fibrosis. We previously demonstrated that the expression of major vault protein (MVP), a key component of the vault complex, was increased in proximal renal tubular epithelial cells in CKD patients. MVP has been shown to mediate diverse cellular responses including resistance to chemotherapy in malignant cells, and inhibition of NFκB-signaling mediated inflammation in atherosclerosis. The role of MVP in CKD remains to be defined.

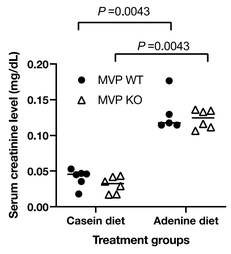
Methodology

CKD was induced in wild-type (WT) and MVP knockout (KO) mice by feeding with casein-based chow containing 0.2% adenine for 8 weeks. Mice fed with casein-based chow served as controls. Mice were sacrificed, serum collected and kidneys harvested for immunohistochemistry, qPCR and flow cytometry analysis. MVP-deficient HK-2 cells were generated and stimulated with TNF-α. Non-transfected HK-2 cells served as control.

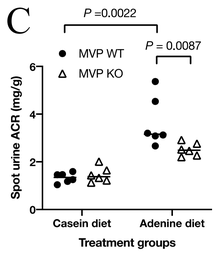
Results

A



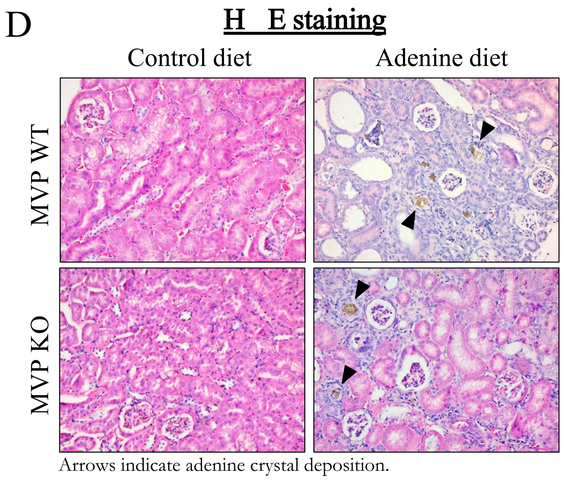


C

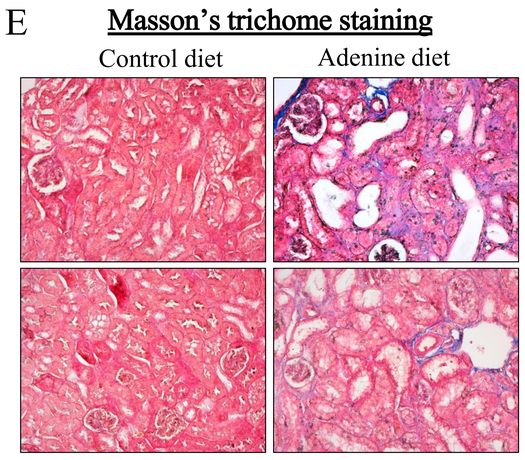


- Serum urea and creatinine levels were similar in WT and MVP KO mice with CKD (Fig. A & B). Spot urine albumin-to-creatinine ratio (uACR) (Fig. C) was significantly lower in MVP KO mice with CKD compared with WT mice.

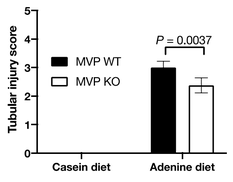
D



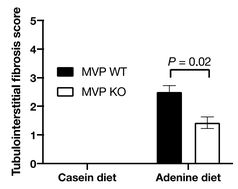
E



Arrows indicate adenine crystal deposition.

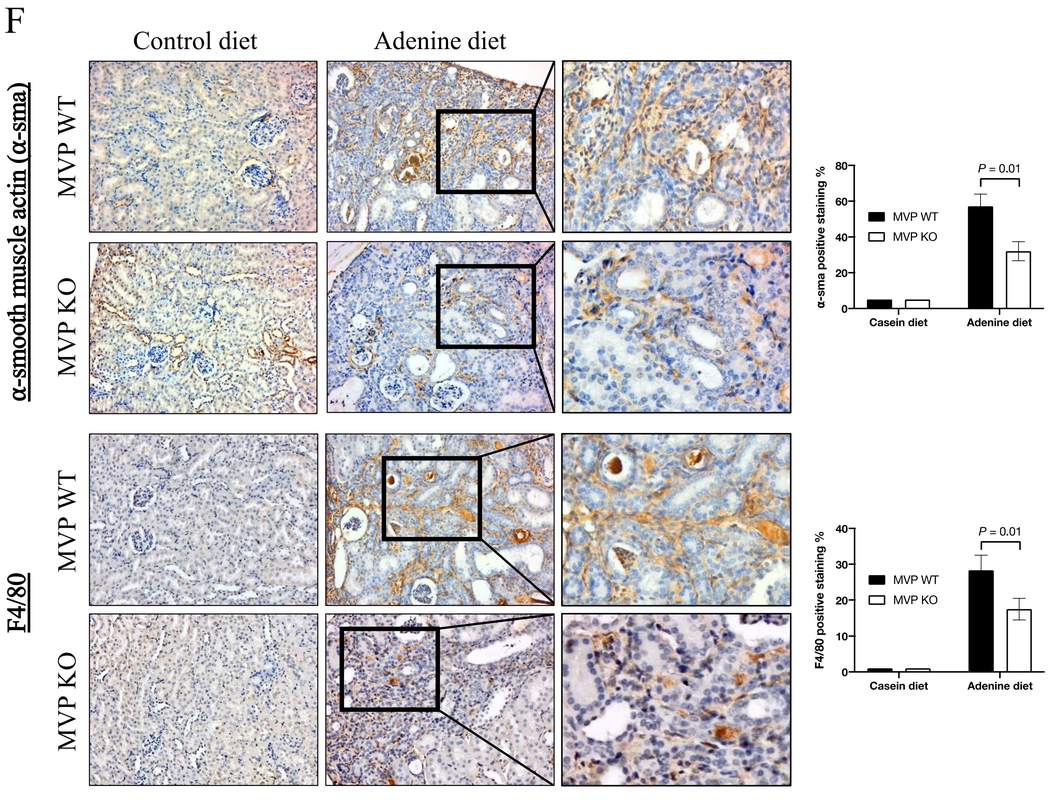


Scoring system			
0	No damage	3	51% - 75% area
1	2% - 25% area	4	76% - 100% area
2	26% - 50% area		



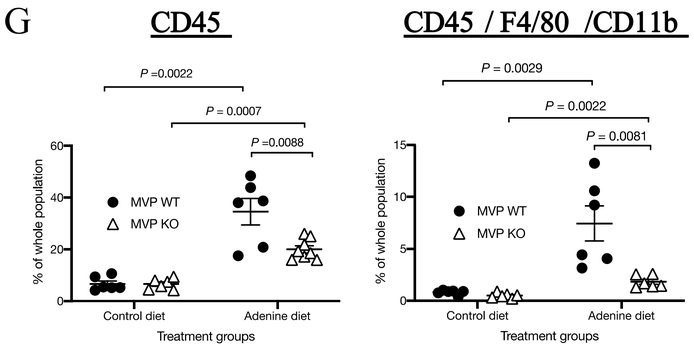
- In adenine-treated WT mice, H&E (Fig. D) and Masson's trichrome staining (Fig. E) showed tubulo-interstitial abnormalities including tubular atrophy, increased immune cell infiltration and interstitial collagen deposition. These histopathological changes were significantly reduced in MVP KO mice.

F



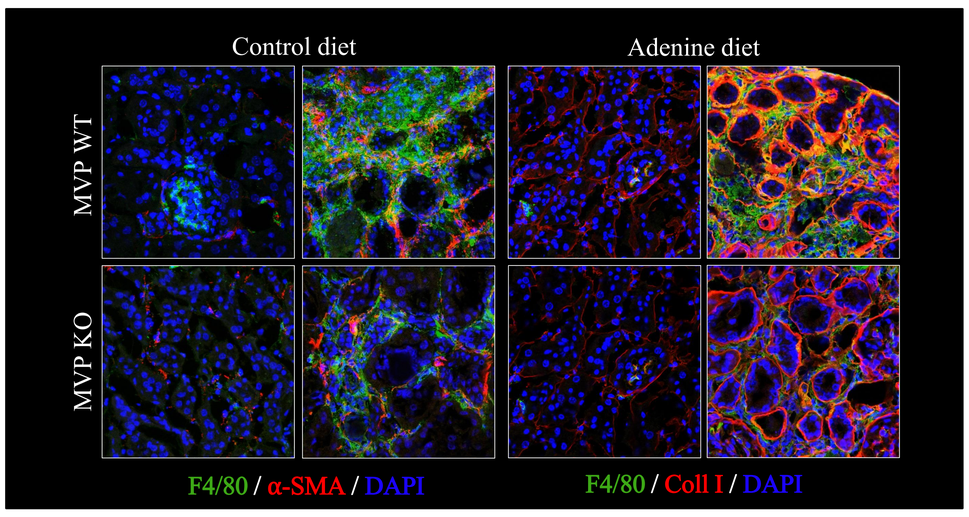
- WT mice with CKD showed more alpha-smooth muscle actin (α-sma) positive staining, indicative of myofibroblast recruitment, in the tubulointerstitium compared with MVP KO mice (Fig. F).
- Staining for F4/80, a macrophage marker, was more pronounced in adenine-treated WT mice compared with MVP KO mice, suggesting less macrophages infiltration in MVP KO mice. (Fig. F).

G



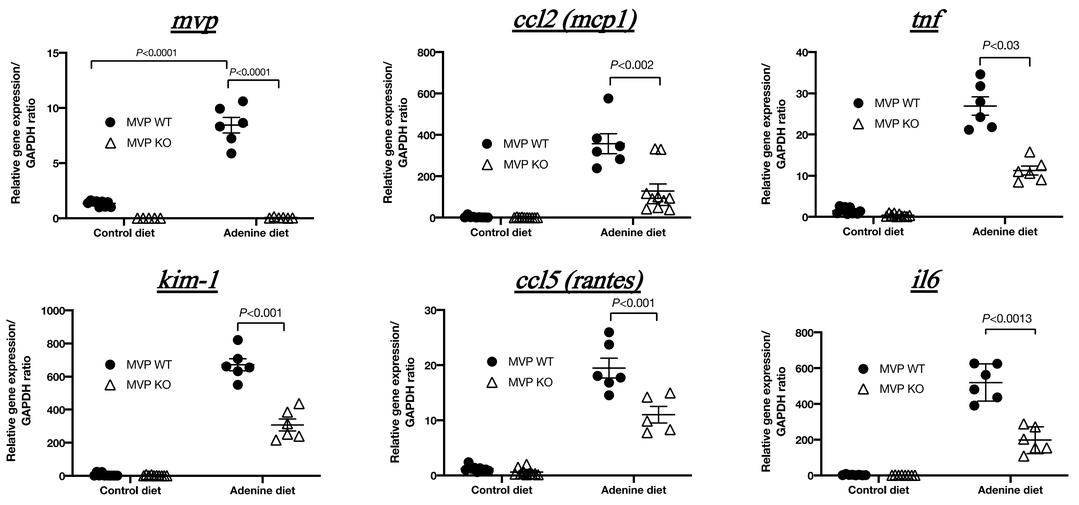
- Flow cytometry analysis showed more $CD45^+$ cells and a higher percentage of $CD45^+/F4/80^+/CD11b^+$ triple positive cells in WT mice with CKD compared with MVP KO (Fig. G).

H

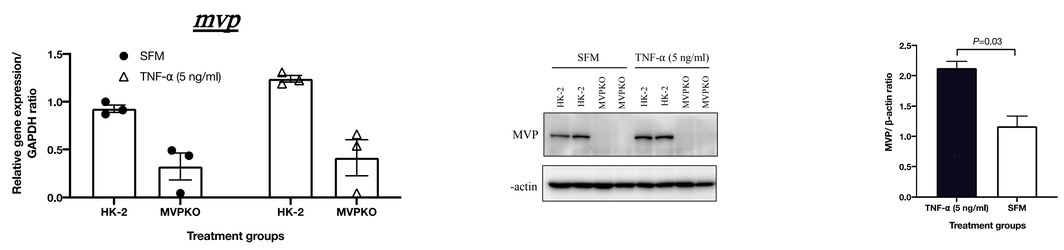


- There were more colocalization of F4/80 with α-sma and collagen I positive staining in WT mice with CKD compared with MVP KO mice (Fig. H), suggesting that subsets of macrophages may undergo macrophage-to-myofibroblast transition and contribute to kidney fibrosis.

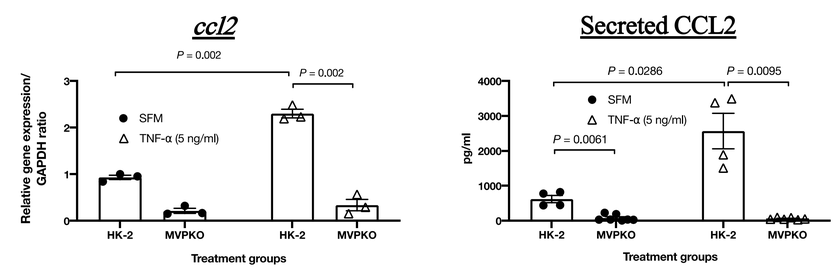
I



- MVP mRNA level was significantly increased in adenine-treated WT compared with control WT mice (Fig. I). mRNA level of Kim-1, a marker for tubular injury was higher in WT mice with CKD compared to MVP KO mice, suggesting that tubular injury in MVP KO mice is less severe than in WT mice.
- Gene expression levels of *ccl2*, *ccl5*, *tnf* and *il-6* were significantly lower in MVP KO mice with CKD compared to WT mice suggesting that increased in MVP expression contributes to kidney inflammation (Fig. I).



K



- At the transcription level, the MVP knockdown efficiency in MVP-deficient HK-2 cells was more than 60%, whereas MVP was completely silenced at the protein level (Fig. J).
- Exogenous TNF-α induced MVP expression (Fig. J), and this was accompanied by an increase in ccl2 gene expression and protein secretion in non-transfected HK-2 cells (Fig. K). The effect of TNF-α on ccl2 gene expression and secretion was significantly reduced in MVP-deficient HK-2 cells (Fig. K).

Conclusion

Our data suggest that MVP plays a role in the pathogenesis of CKD, possibly through its effects on inflammatory and fibrotic processes involving renal tubular epithelial cells.

Acknowledgement

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