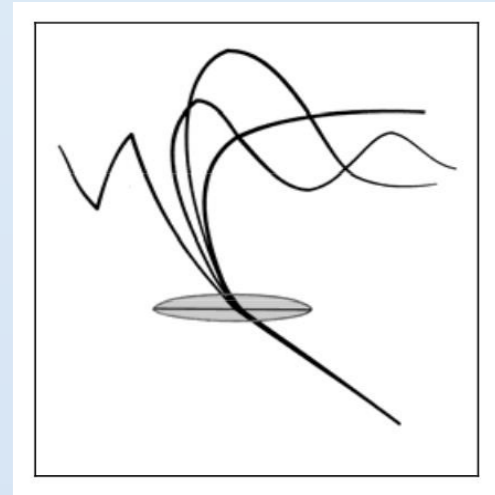


Differences in renal microscopic morphology between wild-type and *Tff3* knock-out mice



Bijelić Nikola¹, Martina Firić², Edi Rođak¹, Tatjana Belovari¹, Šešelja Kate³, Bazina Iva³, Mirela Baus Lončar³

¹Department of Histology and Embryology, Faculty of Medicine, J. Huttlera 4, 31000 Osijek, Croatia; ²University Graduate Study of Medical Laboratory Diagnostics, Faculty of Medicine, J. Huttlera 4, 31000 Osijek, Croatia; ³Institute Ruđer Bošković, Bijenička 34, 10000 Zagreb, Croatia

Introduction

Tff3 peptide is a mucosal protein involved in protection and restoration of the mucosal barrier, especially in the intestines. It is also found in the kidney and is believed to facilitate the repair of kidney tubular epithelium injury. Tff3 peptide expression is also related to some renal pathological conditions, such as renal cancer and chronic kidney disease. The objective of this study was to investigate the effect of Tff3 peptide deficiency on histomorphometric properties of kidney tissue using wild-type mice and *Tff3* knock-out mice.

Materials & methods

Male mice (background C57Bl6/J//Sv129) deficient of Tff3 protein and appropriate wild type mice were used in the study (N=5 each group). Kidneys were harvested, fixed in 4% paraformaldehyde and paraffin-embedded. Blocks with kidney tissue were cut (6 μm thick) and slides were stained using PAS method. Digital photographs were taken (5 images of cortex and 3 images of medulla for each animal) and measured using FIJI software. In the cortex, glomeruli, proximal convoluted tubules (PCT) and distal convoluted tubules (DCT) were measured, and in the medulla, collecting ducts (CD) were measured (Fig. 1). Arithmetic means were obtained for each sample and used for further analysis.

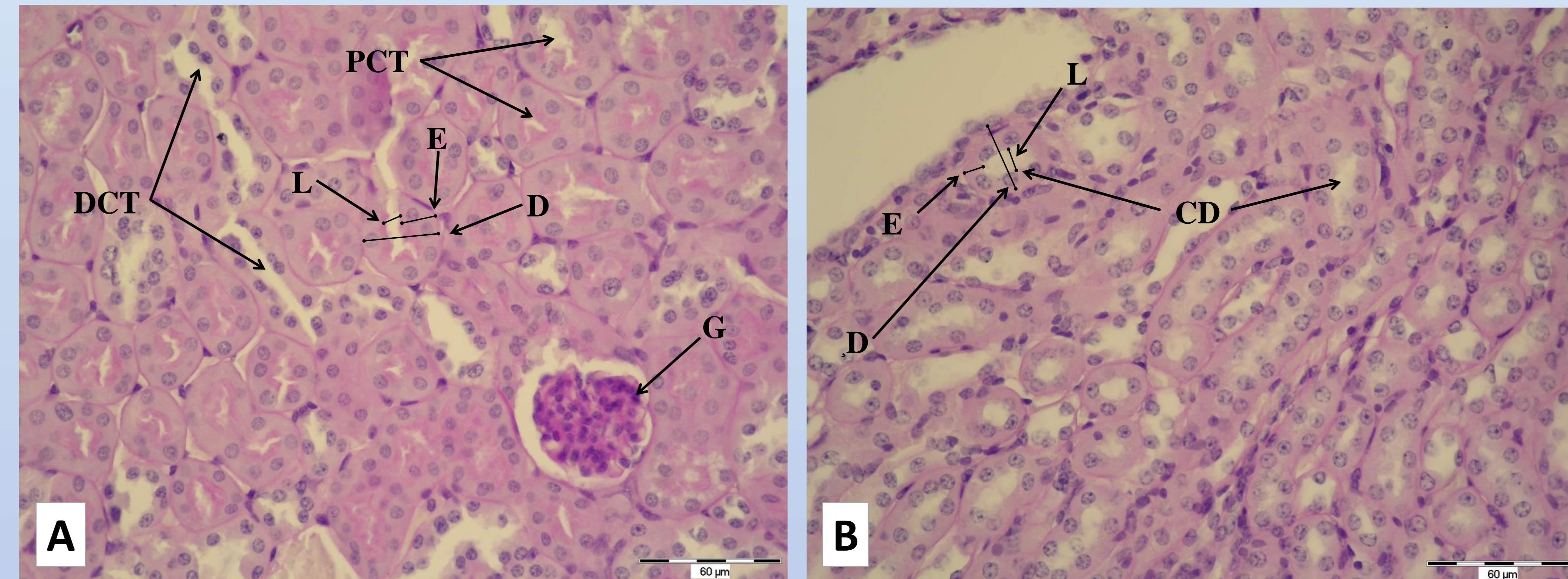


Figure 1. Cortex and medulla measurements. A – cortex; B – medulla. G – glomerulus, DCT – distal convoluted tubule, PCT – proximal convoluted tubule, E – height of epithelium, L – lumen diameter, D – tubule diameter, CD – collecting duct. Scale bar: 60 μm.

Table 1. Results of cortical morphology measurements. Table 2. Results of medullar morphology measurements

CORTEX	Median (interquartile range)		p*
Measured value	Wild-type mice	<i>Tff3</i> knock-out mice	
Glomerular area	3331,56 (2100,30 - 5255,77)	3779,67 (2609,91 - 3992,60)	> 0,95
PCT diameter	29,75 (29,42 - 35,22)	39,32 (37,23 - 40,15)	0,03
Height of PCT epithelium	12,20 (11,23 - 13,70)	13,77 (13,27 - 15,1)	0,15
PCT lumen diameter	3,95 (3,67 - 4,24)	5,35 (4,31 - 6,43)	0,03
DCT diameter	26,73 (25,03 - 29,49)	25,63 (23,27 - 28,95)	0,84
Height of DCT epithelium	10,41 (9,17 - 10,60)	10,33 (9,12 - 11,07)	0,95
DCT lumen diameter	6,28 (4,64 - 7,17)	5,41 (5,21 - 7,20)	0,95

*Mann-Whitney U test

MEDULLA	Median (interquartile range)		p*
Measured value	Wild-type mice	<i>Tff3</i> knock-out mice	
CD diameter	26,02 (23,88 - 27,77)	24,09 (23,65 - 25,03)	0,29
Height of CD epithelium	8,38 (7,48 - 9,23)	8,46 (7,48 - 8,79)	0,73
CD lumen diameter	8,31 (7,93 - 9,60)	7,93 (7,36 - 8,63)	0,56

*Mann-Whitney U test

Results

Tff3-deficient mice have larger total diameter and lumen width of PCTs compared to wild-type mice. Height of PCT epithelium was larger as well, but not significantly. Significant differences were not found in other cortical histomorphological properties of the examined groups (Table 1). CD morphology was not affected significantly (Table 2).

Conclusion

All measured parameters of PCTs were increased in *Tff3* knock-out mice, 2 out of 3 significantly. This increase in PCT size might point to proximal tubule hypertrophy in *Tff3* knock-out mice. It is interesting that PCT is one of the main sites of Tff3 peptide expression in the kidney. Since Tff3 peptide is known to be involved in epithelial homeostasis and protection, it is possible that deletion of *Tff3* gene affects the physiology of PCT. In order to confirm this assumption additional studies are needed (including total renal volume and kidney weight measurement, serial kidney sections, studies on mice of different age and studies after pharmacological challenge of kidney function, e.g. using furosemide).