

Chemokines and trefoil factor peptides in patients suffering from chronic kidney disease

Doctoral thesis at the Medical University of Vienna
for obtaining the academic degree „Doctor of Philosophy“

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with additional help of: Claus Krenn^{2,4}, Georg Roth²

Cooperating Departments:

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- RAIC Laboratory 13C^{1,2}
- Department of Thoracic Surgery³
- Division of Nephrology and Dialysis, Department of Medicine III
- Dept. of Anaesthesia, General Intensive Care and Pain Management⁴

Chronic kidney disease

= the clinical manifestation of various renal diseases with comparable evolvement of symptoms of kidney failure.

Defined as **decreased renal function** ($\text{GFR} < 60 \text{ml/min/1.73m}^2$ body-surface) or/and **presence of kidney damage** (albumin/creatinine Ratio $>30 \text{ mg/g Creatinine}$) for a minimum of three months and/or by a **history of kidney transplantation**

(KDIGO 2012 Clinical Practice Guideline for the Evaluation and Management of Chronic Kidney Disease. *Kidney Inter.* 2013;3(1):150).

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➤ **continuously increasing number of affected persons**

15 % of US population;
patients needing kidney replacement therapy doubled within 10 years

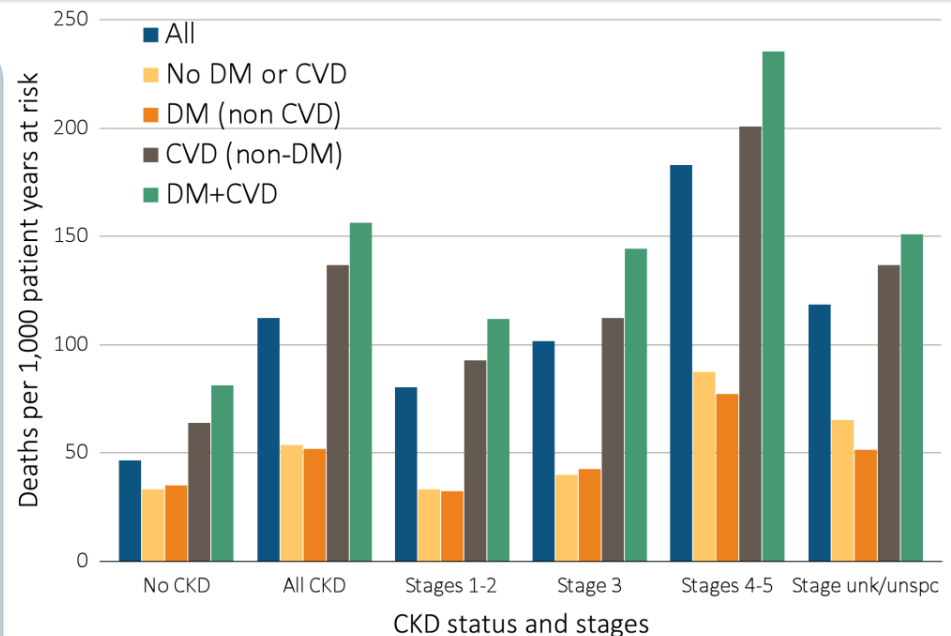
➤ **poor outcome**

mortality rates 50 % higher than other patients

➤ **enormous impact on healthcare costs**

annual US Medicare expenditures: \$87 billion

(Saran R, et al; Am J Kidney Dis. 2015;66(1 Suppl 1):Svii, S1-305.)



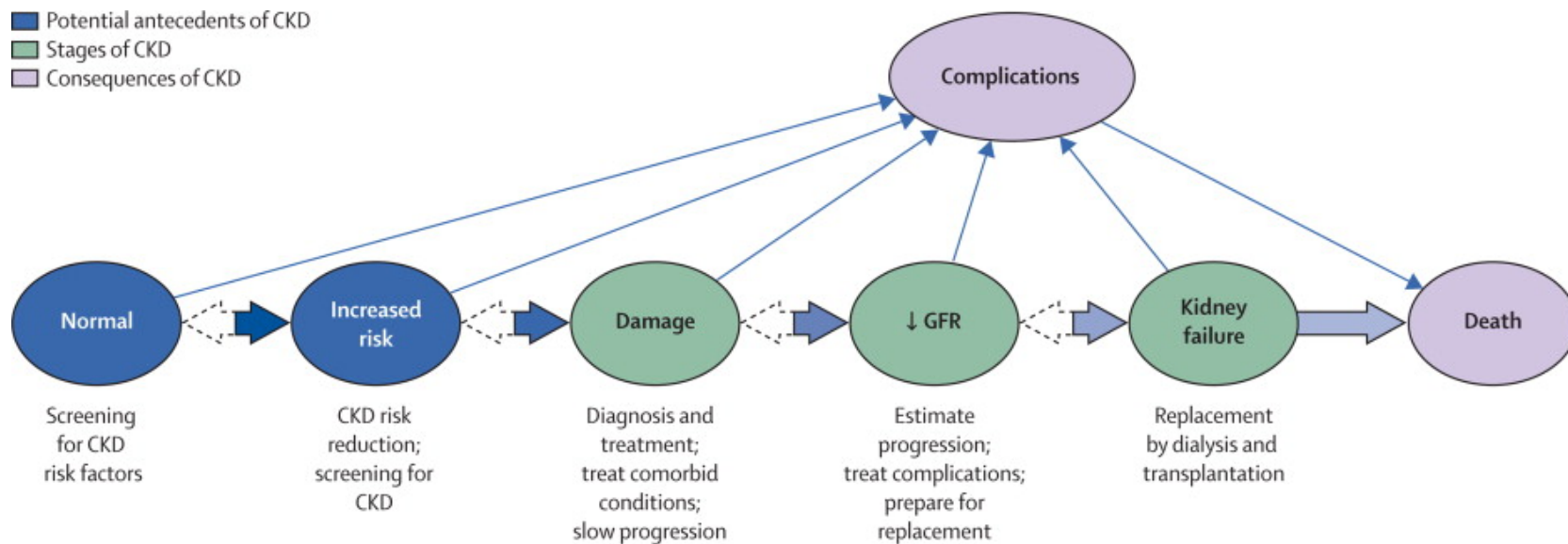
All-cause mortality rates (per 1,000 patient years at risk) for patients (> 65years), by cardiovascular disease and diabetes mellitus, CKD status, and stage in 2014 (2016 Annual Data Report, Vol 1, CKD, Ch 3)

Natural course:

progression to end stage renal disease (ESRD), necessitating kidney replacement therapy

Further major outcomes:

development of complications caused by the impaired kidney function
increased risk for cardiovascular disease



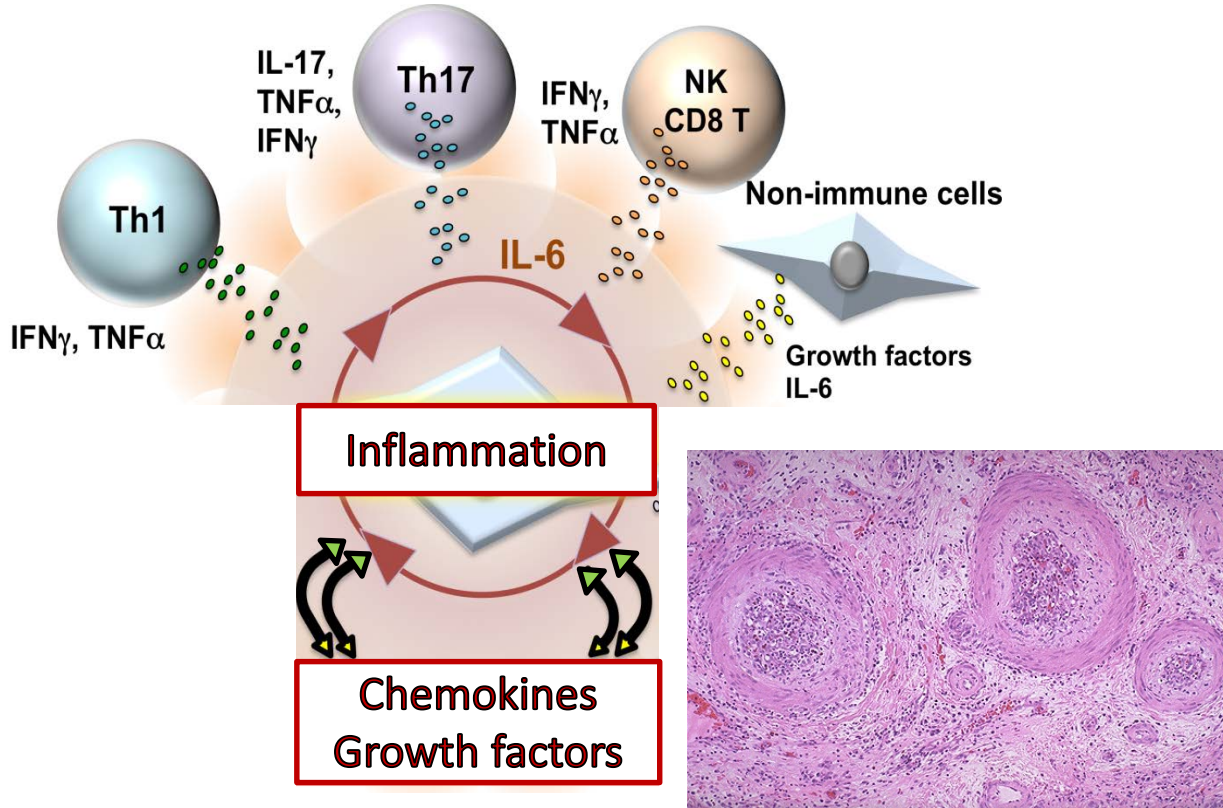
Conceptual model of CKD course and therapeutic strategies.

Complications comprise all complications of CKD (e.g. cardiovascular disease, anaemia, malnutrition, neuropathy and bone disease)

Part 1. Inflammation

Part 2. Counterregulatory mechanisms

Part 1. Inflammation



continuous interstitial inflammation contributes to the persistent loss of renal function and further aggravates the disease progression in a vicious cycle resulting in fibrosis

the activation of different pro-inflammatory cellular pathways:

- upregulation of various cytokines and chemokines
- increased expression of adhesion molecules
- elevated infiltration of inflammatory cells
- release of reactive oxidative species (ROS)

Kamimura D, et al. , The Gateway Reflex, a Novel Neuro-immune Interaction, is Critical for the Development of Mouse Multiple Sclerosis (MS) Models. Chapter 2; ISBN 978-953-51-2657-7, 2016

Image source: <https://library.med.utah.edu/WebPath/RENAHTML/RENAL038.html>

Chemotactic Cytokines Chemokines

1. CC subfamily

Chemokines	Receptors
I-309/CCL1	CCR8
MCP-1/CCL2	CCR2/4/10
MCP-2/CCL8	CCR2
MCP-3/CCL7	CCR1/2
MCP-4/CCL13	CCR1/2
MIP-1 α /CCL3	CCR1/5
MIP-1 β /CCL4	CCR5
MIP-3 α /CCL20	CCR6
RANTES/CCL5	CCR1/3/5
eotaxin-1/CCL11	CCR3
eotaxin-2/CCL24	CCR3
eotaxin-3/CCL26	CCR3
HCC-1/CCL14	CCR1
HCC-2/CCL15	CCR1/3
HCC-4/CCL16	CCR1/3
TARC/CCR17	CCR4
MDC/CCL22	CCR4
ELC/CCL19	CCR7
SLC/CCL21	CCR7
TECK/CCL25	CCR9
CTACK/CCL27	CCR10
MEC/CCL28	CCR10
PARC/CCL16	Unknown
MPIF-1/CCL23	Unknown
vMIP-II	Multiple

2. CXC subfamily

Chemokines	Receptors
IL-8/CXCL8	CXCR1/2
GCP-2/CXCL6	CXCR1/2
NAP-2/CXCL7	CXCR2
ENA-78/CXCL5	CXCR2
GRO α /CXCL1	CXCR2
GRO β /CXCL2	CXCR2
GRO γ /CXCL3	CXCR2
PF4/CXCL4	Unknown
IP-10/CXCL10	CXCR3
Mig/CXCL9	CXCR3
I-TAC/CXCL11	CXCR3
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BCA-1/CXCL13	CXCR5
CXCL16	CXCR6
BRAK/CXCL14	Unknown

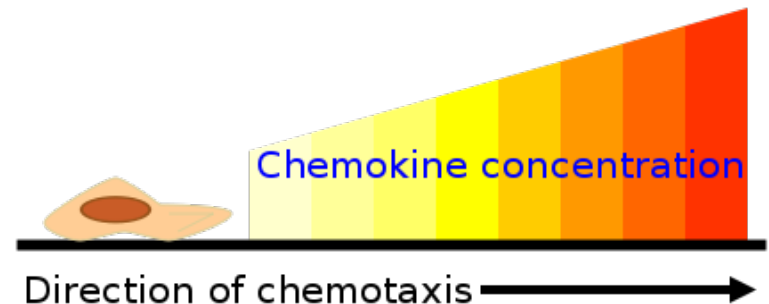
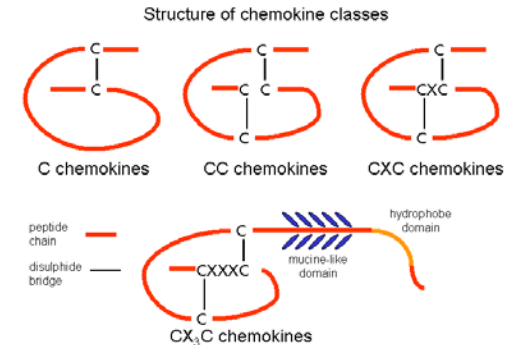
3. XC subfamily

Chemokines	Receptors
Lymphotactin/XCL1	XCR1
SCM-1b/XCL2	XCR1

4. CX3 subfamily

Chemokines	Receptors
Fractalkine/CX3CL1	CX3CR1

grouped according to the **molecular structure** of a conserved four-cysteine motif, present near the N-terminus; bind to G protein-coupled receptors



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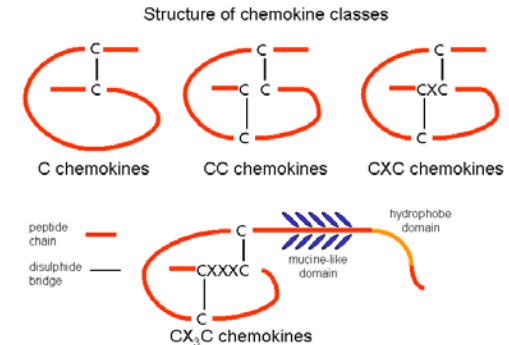
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Homeostatic: for basal leukocyte migration, organize the microarchitecture of secondary lymphoid organs, regulate leukocyte homing, facilitate the processes of cross talk between leukocytes (e.g. CCL14, CCL19, CCL20, CCL21, CCL25, CCL27, CXCL12 and CXCL13. no strict classification).

Inflammatory: formed on pro-inflammatory stimuli, such as IL-1, TNF-alpha, LPS, or viruses, attracting immune cells to the site of inflammation. (e.g. CXCL-8, CCL2, CCL3, CCL4, CCL5, CCL11, CCL20, CXCL10)

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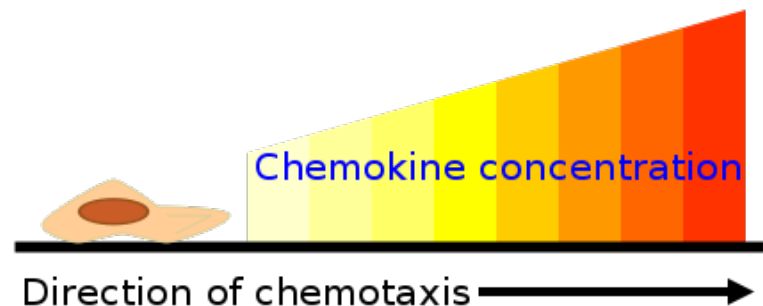
4. CX3 subfamily

Chemokines	Receptors
Fractalkine/CXCL3	CX3CR1

Inflammatory: formed on pro-inflammatory stimuli, such as IL-1, TNF-alpha, LPS, or viruses, attracting immune cells to the site of inflammation.

- Some bind to **multiple receptors of the same group**
- Some are **antagonists of receptors of other groups**
- most receptors bind more than one ligand
- variable affinity
- functionally different cell types can express the same chemokine receptors but still differ in the overall pattern of receptors

essential for the exact adjustment of immune response during inflammation



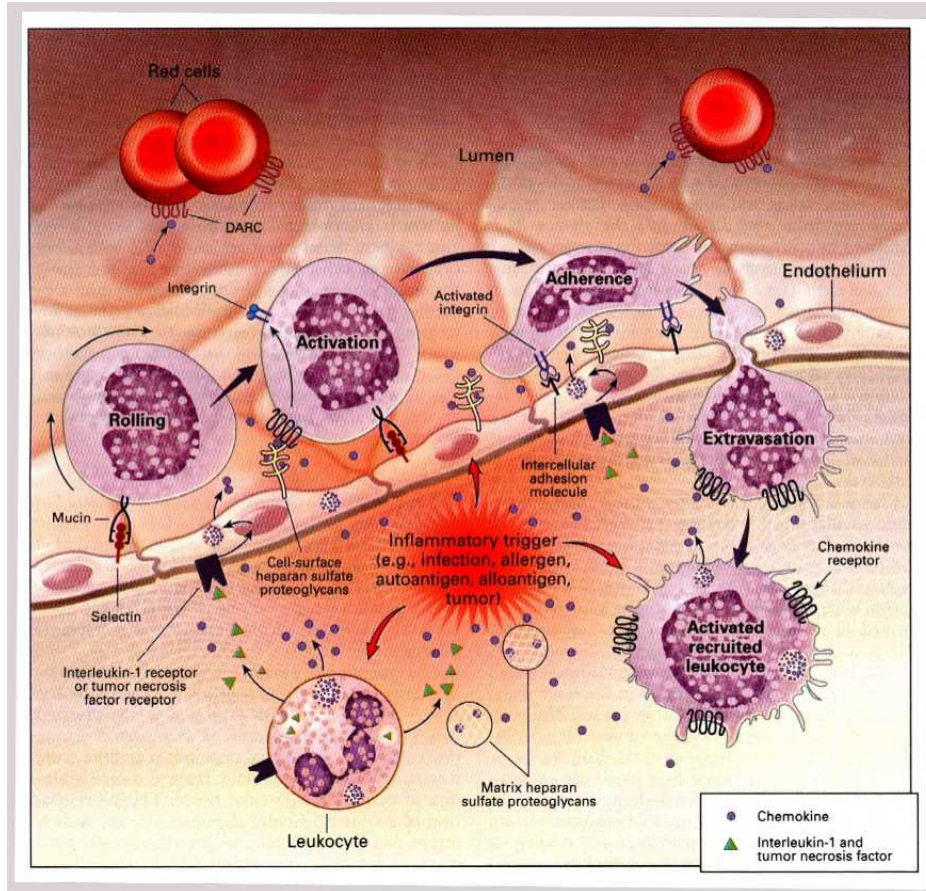
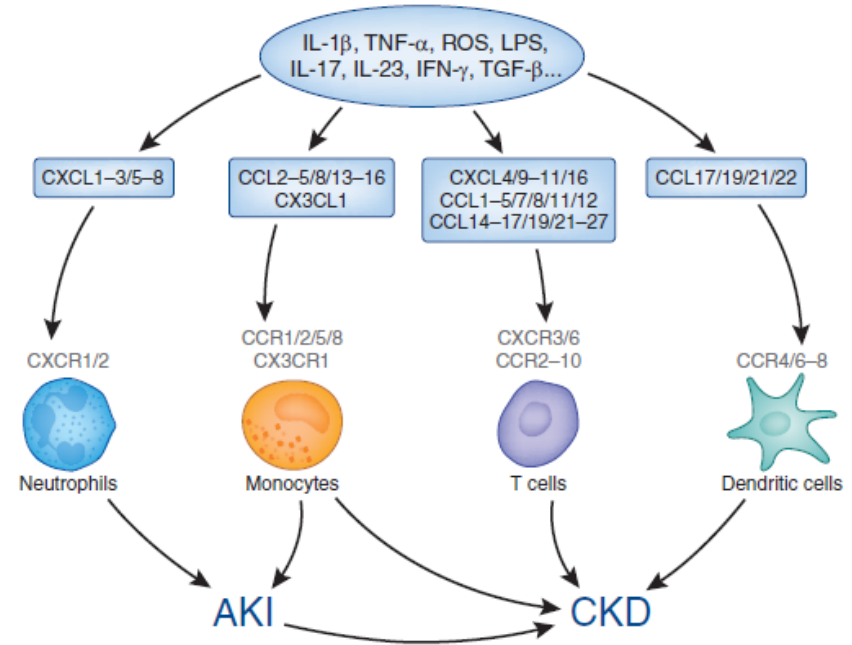
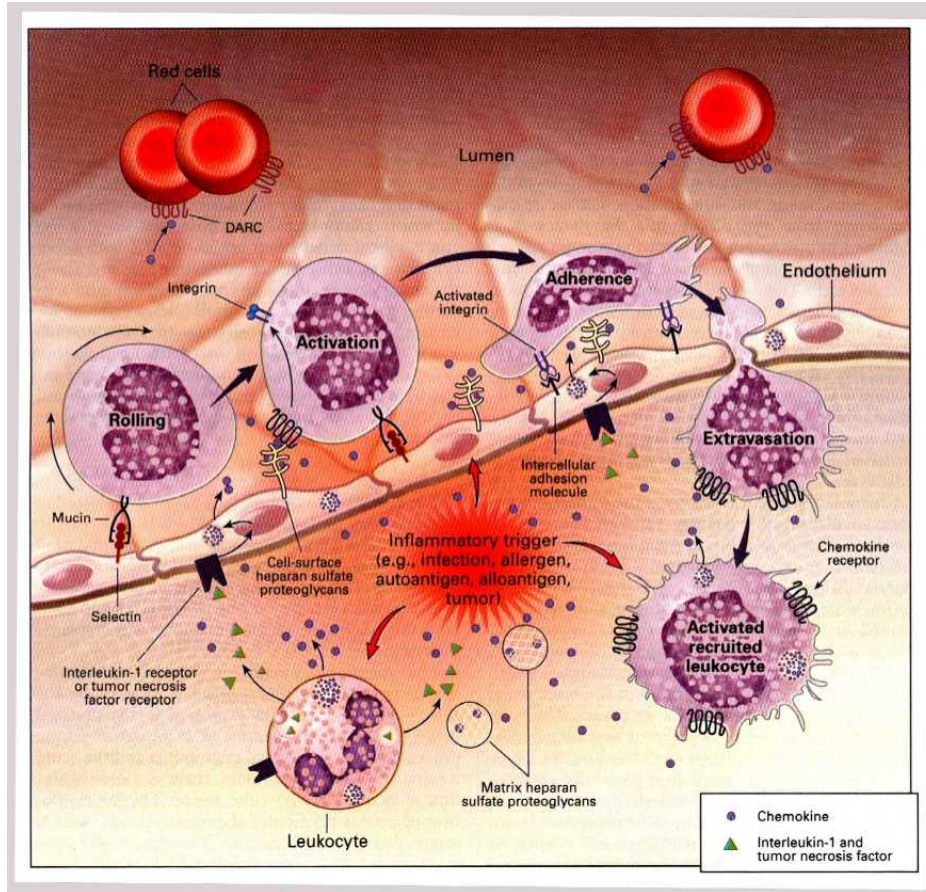


image source:

<http://www.bio.davidson.edu/courses/immunology/chemokinespeech/chemokinetalks.html>



Acute kidney injury (AKI):
Neutrophils and monocytes

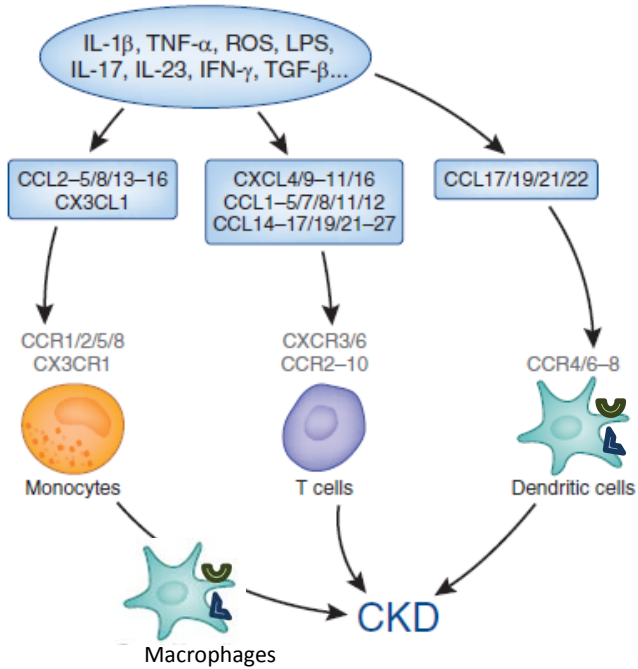
Chronic kidney disease (CKD):
activated macrophages
T cells
dendritic cells

image source:

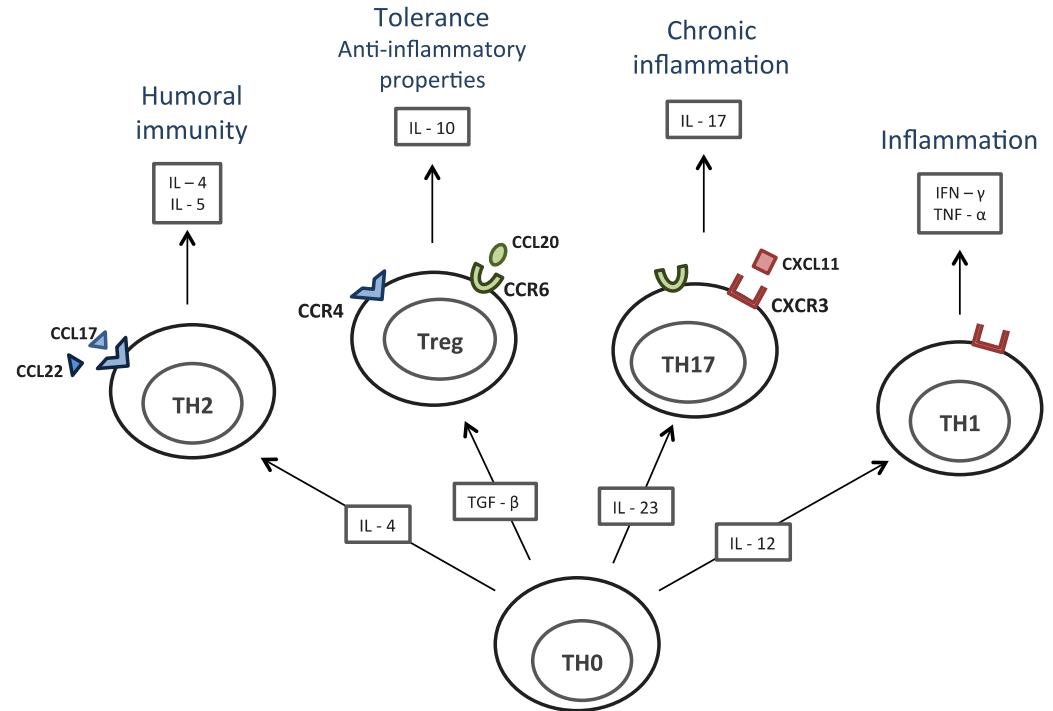
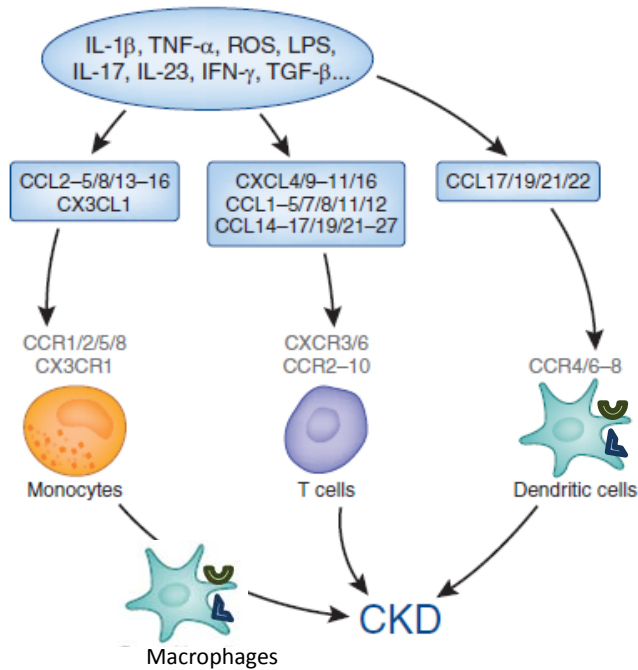
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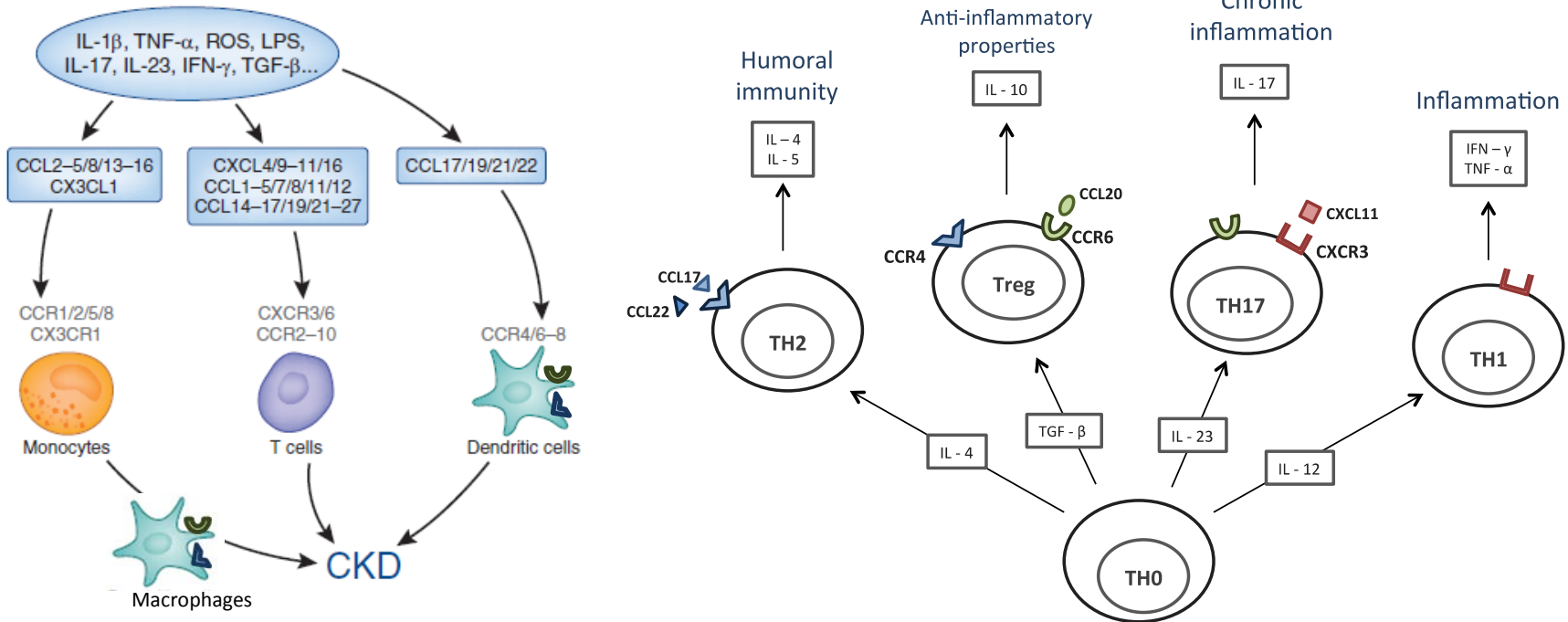
Chung AC, Lan HY. Chemokines in renal injury. J Am Soc Nephrol. 2011;22(5):802-9

Part 1. Inflammation
Analyzed chemokines



Part 1. Inflammation Analyzed chemokines





CCL22/CCL17: blockade of CCL22 \rightarrow no effect in early phase of nephritis but less recruitment of macrophages and **reversion of renal function in later stages** (Garcia GE, et al. Am J Pathol 2003;162:1061-73)

CCL20: increased CCL20 \rightarrow elevated T cell recruitment and **general loss of renal function.**

CCR6- knockout mice \rightarrow **nephritic kidney injury increased**, diminished recruitment of Tregs (Turner JE, et al. J Am Soc Nephrol. 2010;21(6):974-85)

CXCL11: deletion of CXCR3 \rightarrow **decreased renal inflammation** in lupus nephritis and glomerulonephritis (Steinmetz OM, et al. J Immunol. 2009;183(7):4693-704. , Panzer U, et al. J Am Soc Nephrol. 2007;18(7):2071-84.)

1. Patients

Demographic parameters, CKD etiologies, laboratory values	All patients	CKD 1	CKD 2	CKD 3	CKD 4	CKD 5	Controls
N	114	10 (8.8%)	20 (17.5%)	40 (35.1%)	25 (21.9%)	19 (16.7%)	21
Age (y)	59 (19–88)	36 (19–61)	50 (19–80)	63 (23–78)	59 (29–88)	65 (20–81)	32 (21–67)
Gender (male/female)	66/48	7/3	8/12	27/13	15/10	9/10	14/7
Kidney disease							
Glomerulonephritis	33	3	8	9	6	7	
Vascular nephropathy	19	2	—	9	7	1	
Diabetic nephropathy	11	1	—	7	3	—	
Polycystic kidney disease	8	2	—	2	2	2	
Interstitial nephropathy	7	—	4	1	1	1	
Urine stasis	6	—	1	1	2	2	
Nephrectomy	4	—	2	—	—	2	
Carcinoma	4	—	1	2	1	—	
Unknown	21	2	3	9	3	4	

2. Laboratory testing

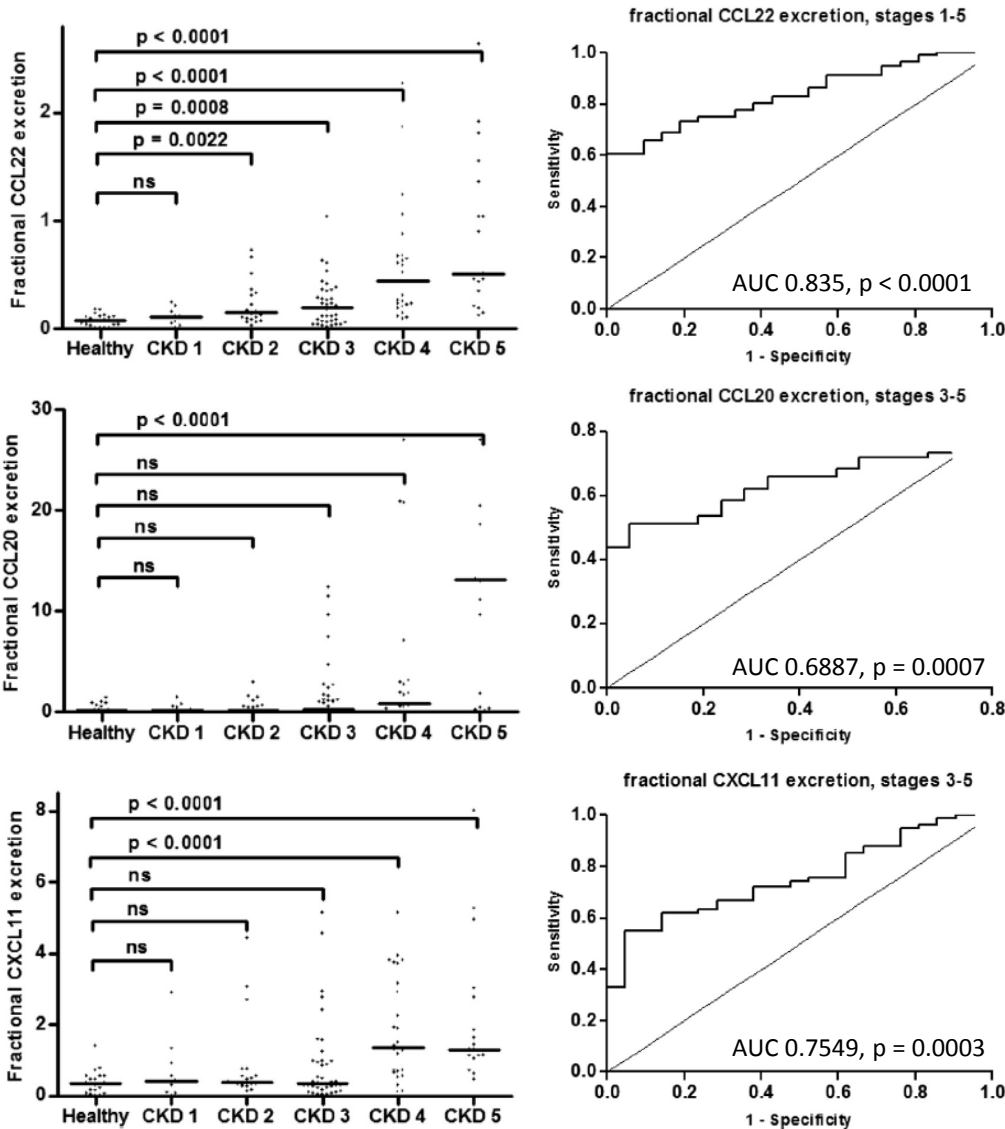


3. Fractional chemokine excretion (%) = 100 x $\frac{\text{urine chemokine} \times \text{serum creatinine}}{\text{serum chemokine} \times \text{urine creatinine}}$

4. Statistical analysis

- Nonparametric Mann-Whitney test to compare CKD stages with controls
- Bonferrony adjustment for multiple comparison (p < 0.01)

Chemokines in CKD: Results



Significant chemokine levels compared to controls:

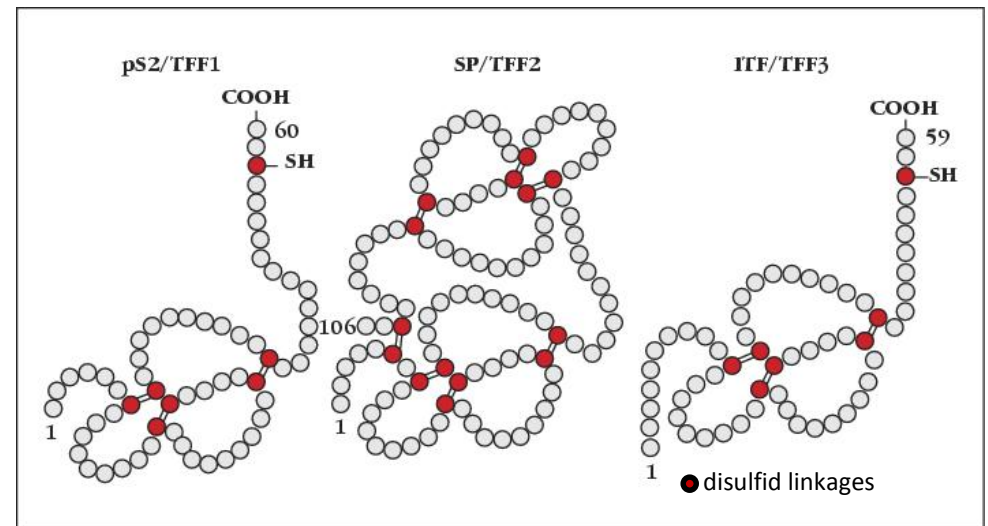
- CCL20 serum CKD 4 ($p < 0.01$)
- CCL20 urine CKD 5 ($p < 0.001$)
- CCL17 serum CKD 5 ($p < 0.01$)
- CXCL11 urine CKD 5 ($p < 0.01$)

Fractional CCL17 excretion ns

Lebherz-Eichinger D, et al. Increased chemokine excretion in patients suffering from chronic kidney disease. *Transl Res.* 2014.

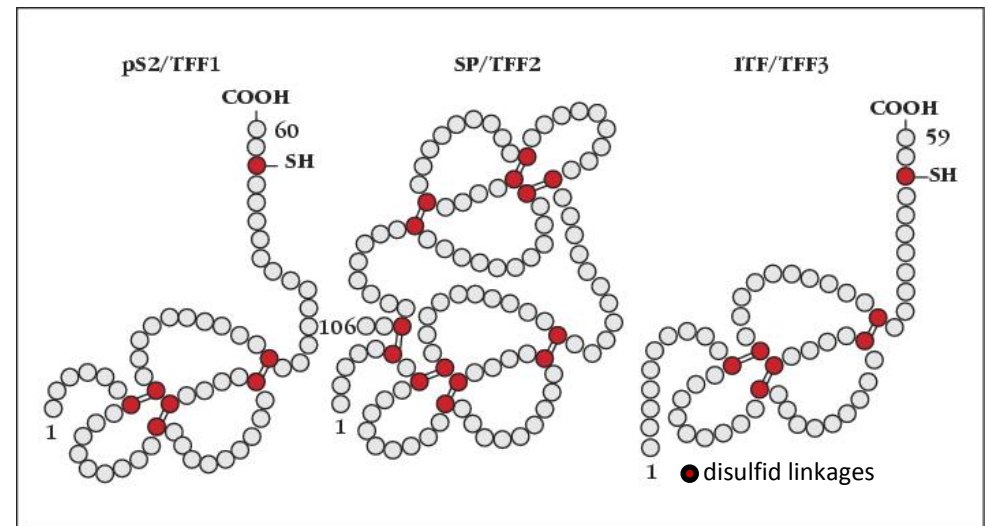
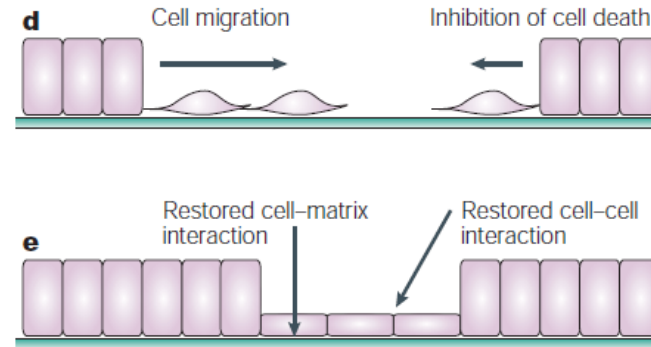
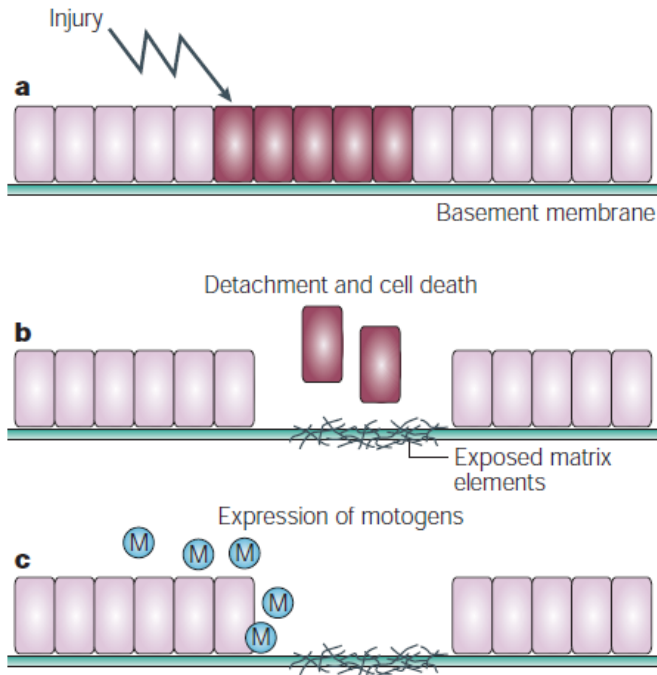
Trefoil Factor (TFF) Peptides

- characterized by their three-leaved shaped pattern of disulphide bonds
- Three members TFF1, TFF2, TFF3
- TFF1/TFF3 form dimers
- Secreted by mucous-producing cells



Taupin D, Podolsky DK. Trefoil factors: initiators of mucosal healing. *Nat Rev Mol Cell Biol.* 2003;4(9):721-32.

Mathelin C, et al. Trefoil factor 1 (pS2/TFF1), a peptide with numerous functions. *Bull du Cancer.* 2005; 92(9): 773-81

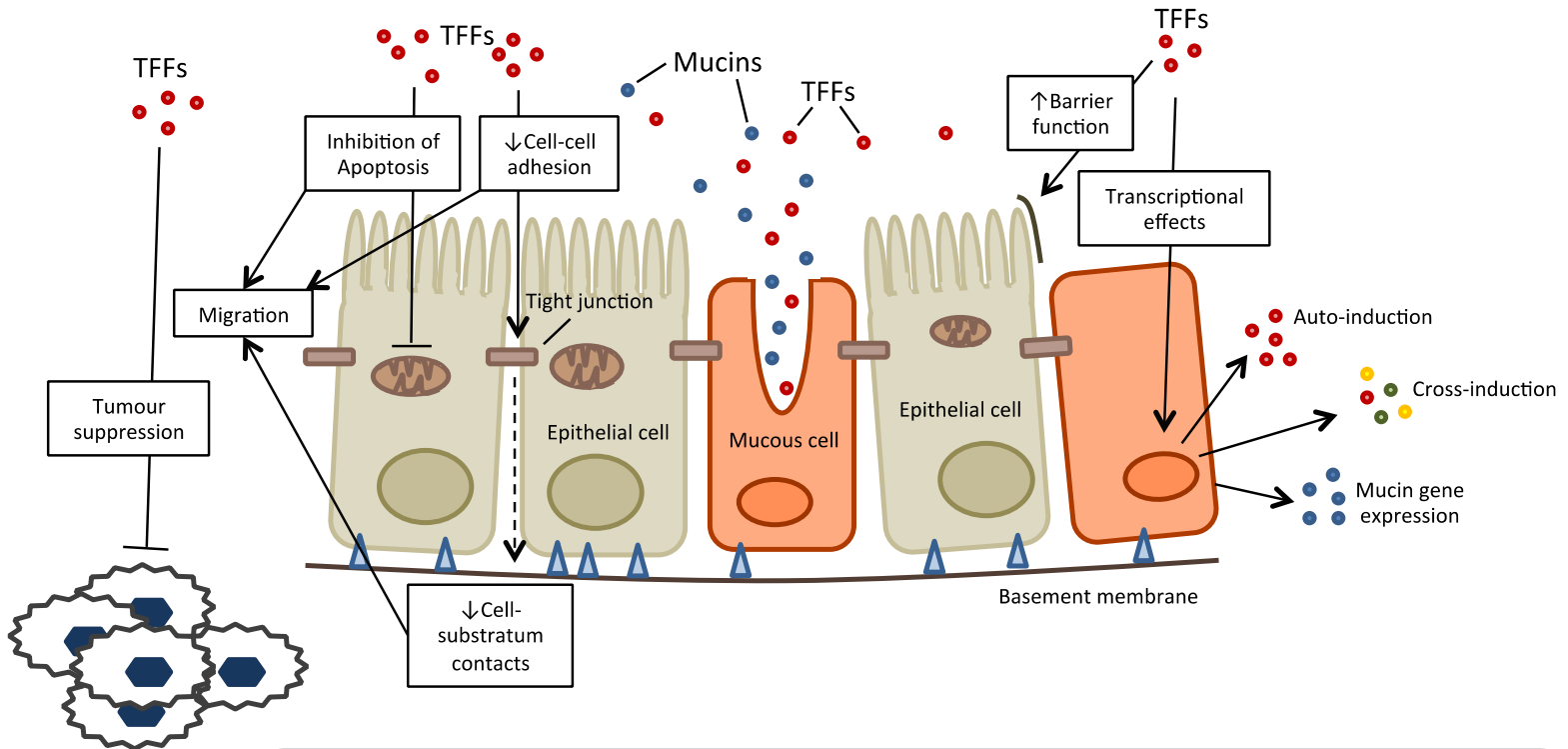


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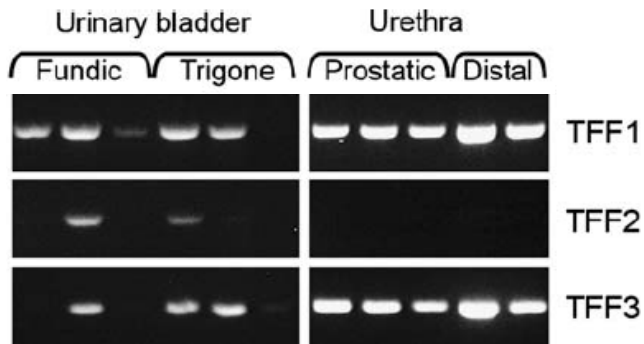
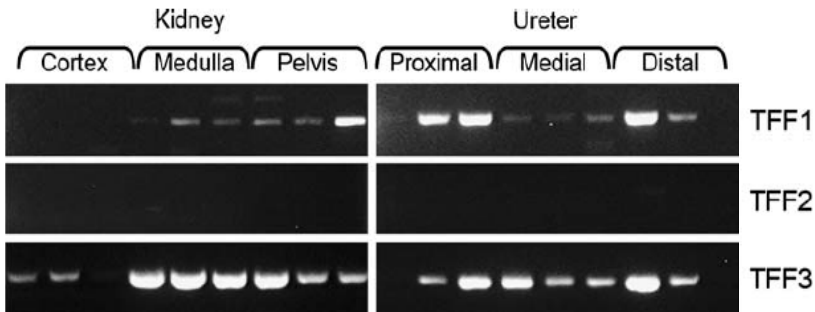
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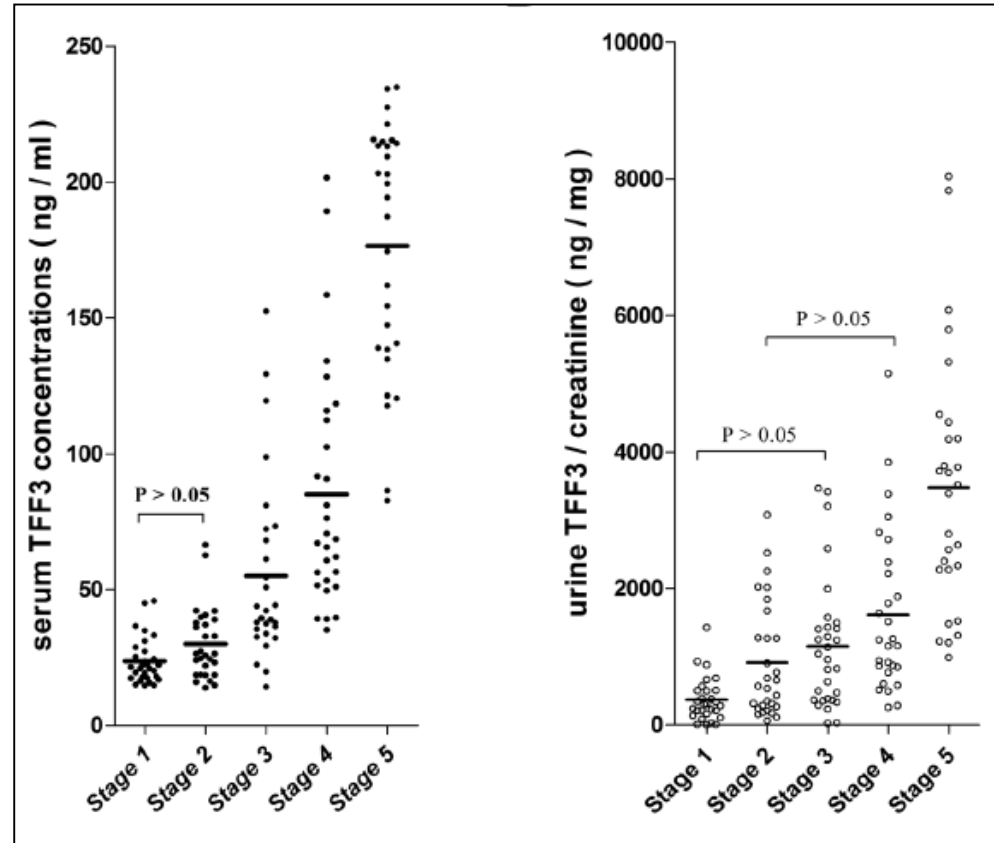
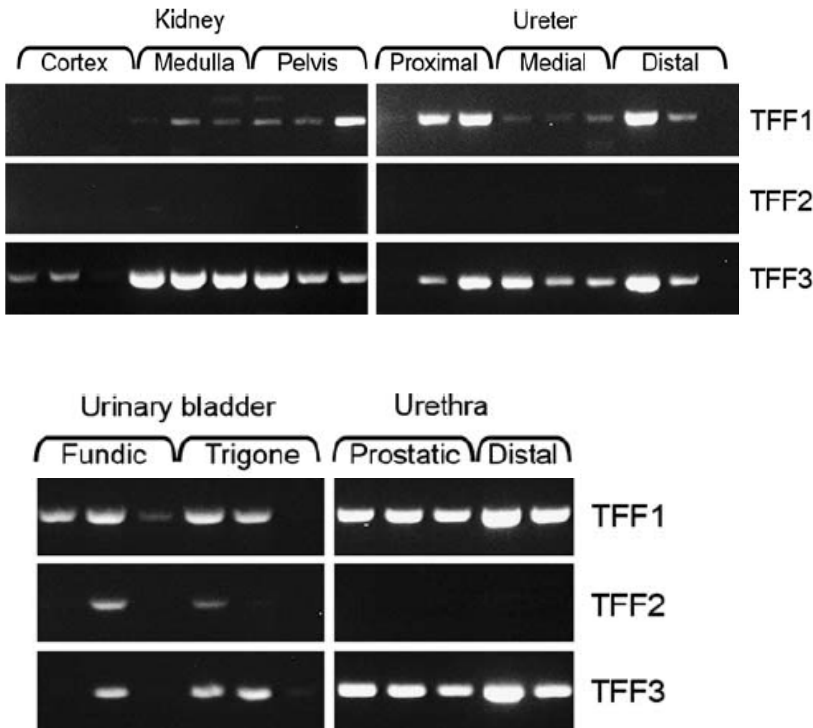
- Epithelial restitution (limiting apoptosis, minimizing cell contacts, angiogenesis)
- Transcriptional effects (tumour suppression, TFF1)
- Induction of mucine gene expression and TFF peptide expression

Part 2. Counterregulatory mechanisms
TFF peptides in the kidneys



RT-PCR from surgical specimens after medically necessary interventions (mainly because of carcinoma)

(Rinnert M, et a. Cell Tissue Res. 2010;339(3):639-47.)



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Urine stasis	6	-	1	1	2	2	
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2. Laboratory testing



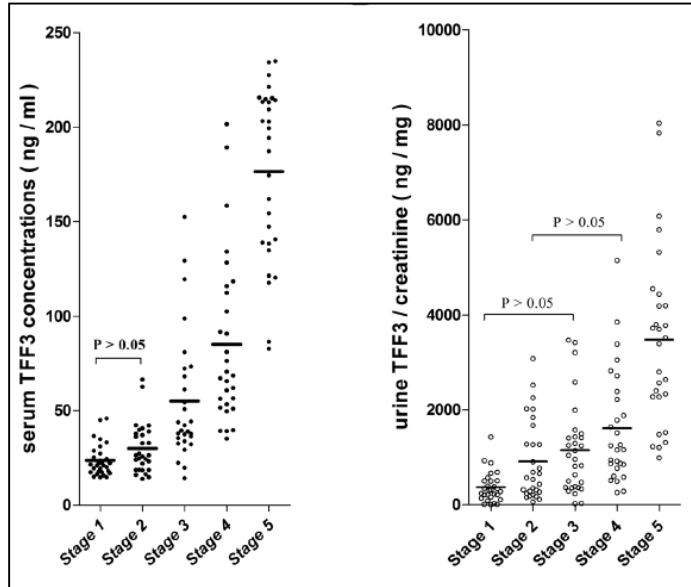
3. Fractional TFF peptide excretion (%) = 100 x $\frac{\text{urine TFF peptide} \times \text{serum creatinine}}{\text{serum TFF peptide} \times \text{urine creatinine}}$

4. Statistical analysis

- D'Agostino-Pearson normality test for Gaussian distribution
- Nonparametric Mann-Whitney test to compare CKD stages with controls
- Bonferroni adjustment for multiple comparison (p < 0.01)

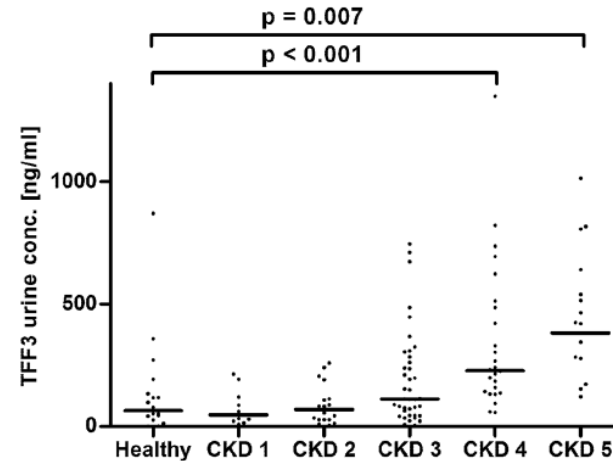
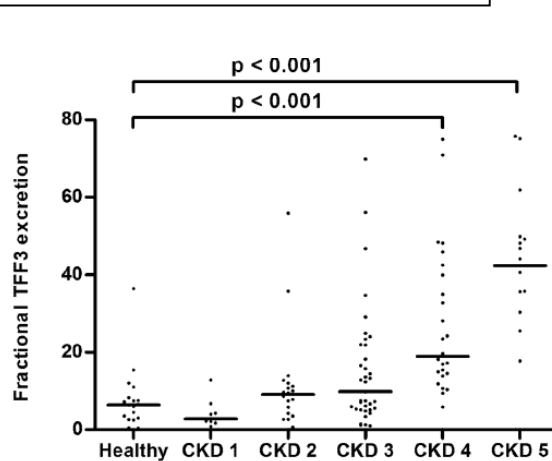
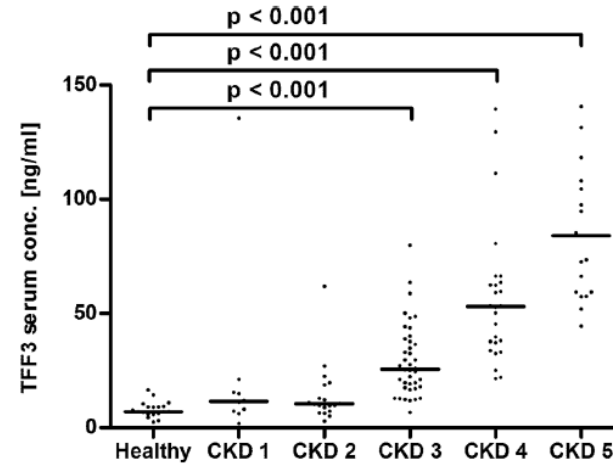
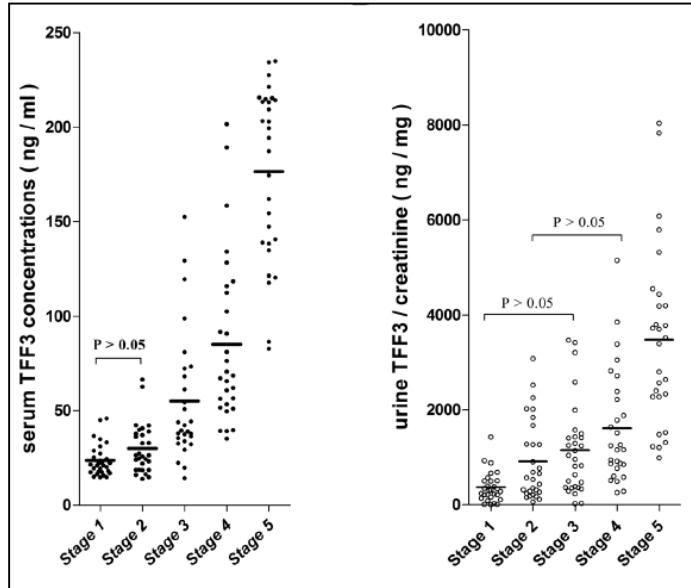
TFF3 in CKD: Results

Du TY, et al. PLoS One. 2014;8(11).



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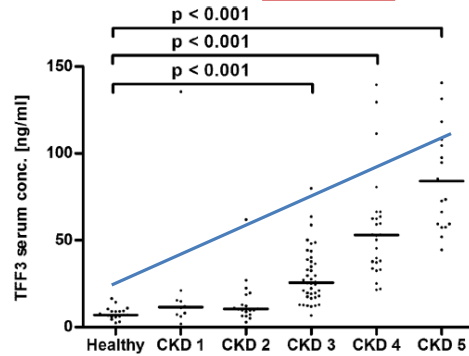
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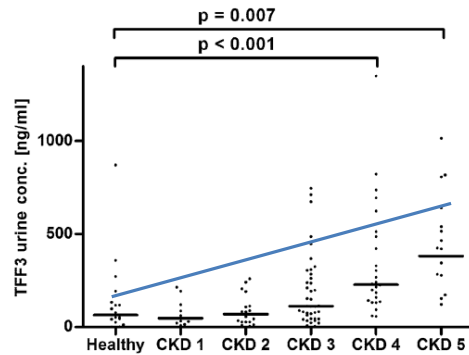
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TFF3

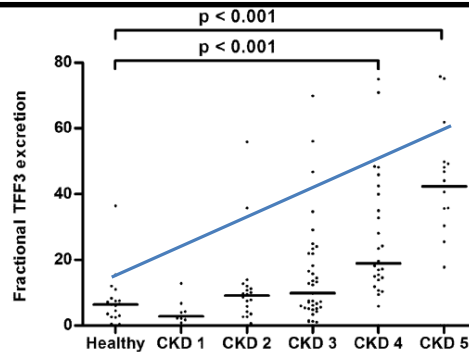
SERUM



URINE



FRACT. EXCR.

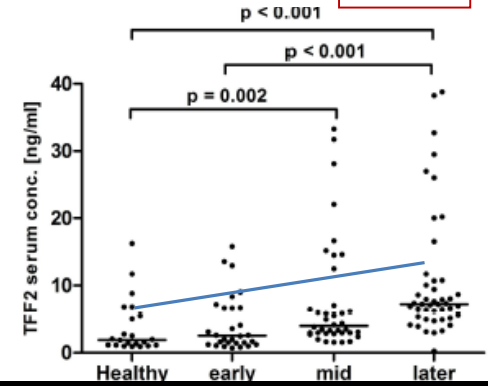
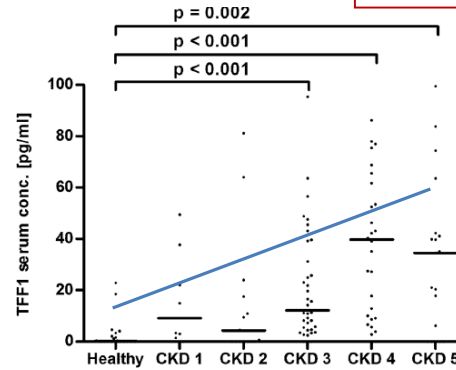
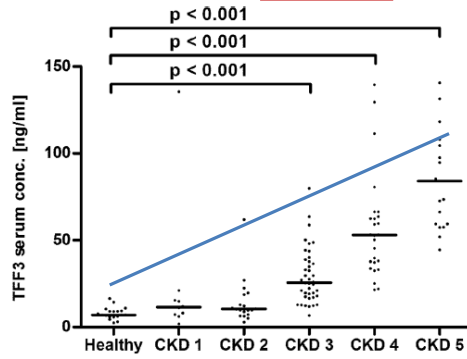


TFF3

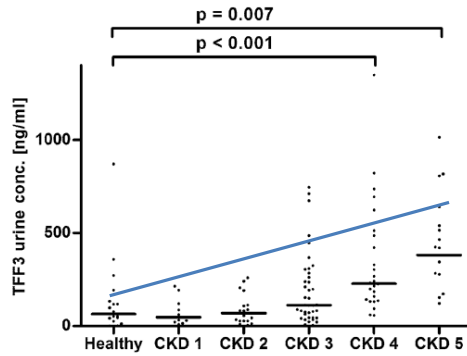
TFF1

TFF2

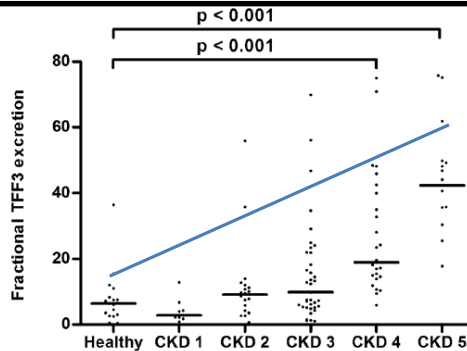
SERUM



URINE



FRACT. EXCR.



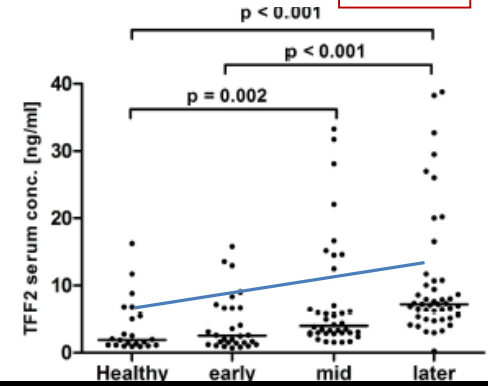
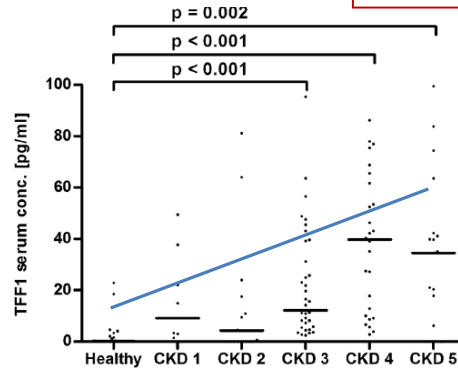
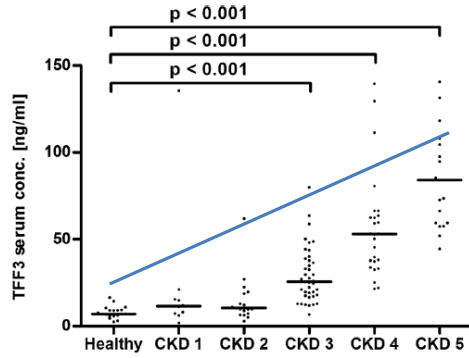
**TFF peptides in CKD:
Results**

TFF3

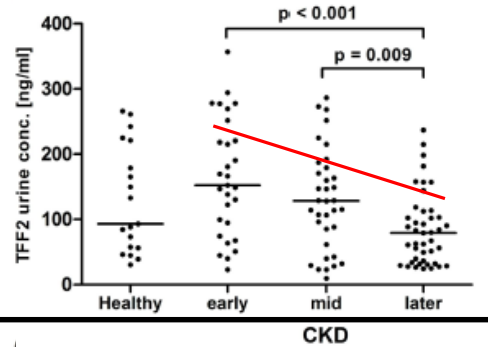
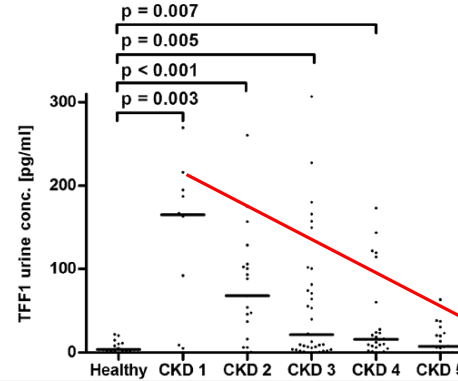
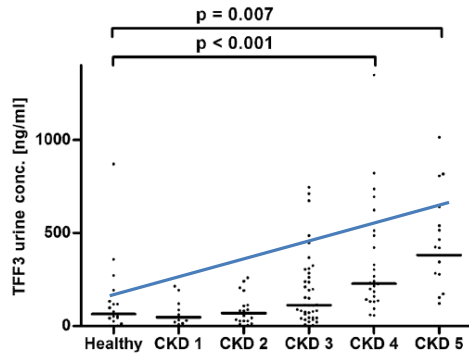
TFF1

TFF2

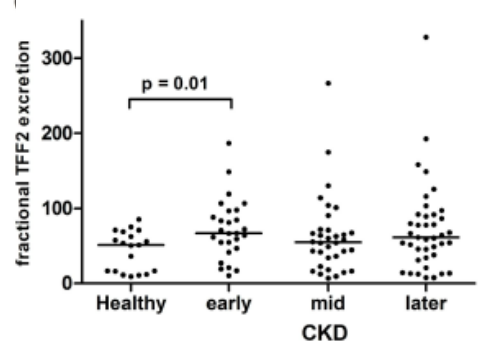
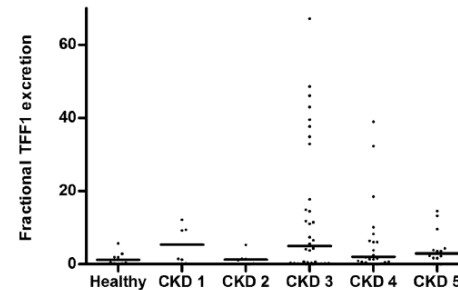
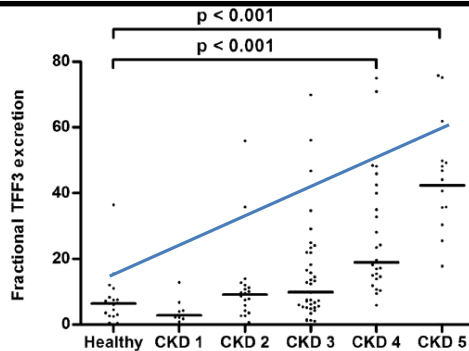
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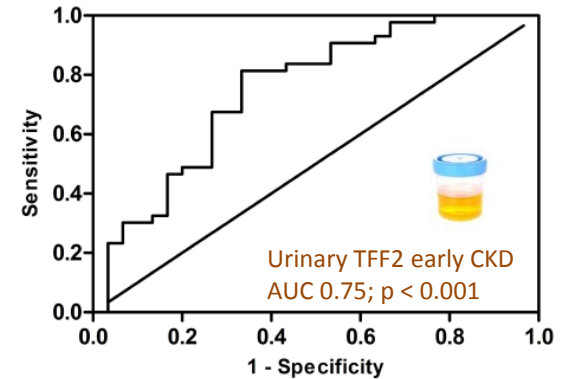
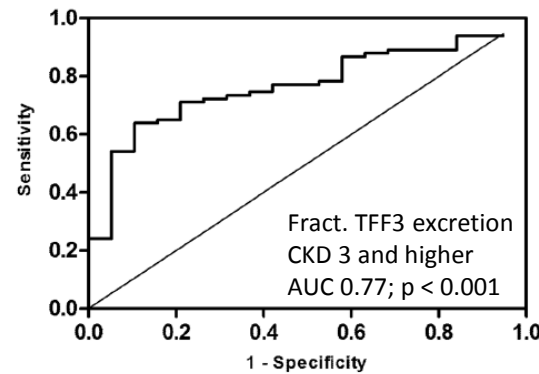
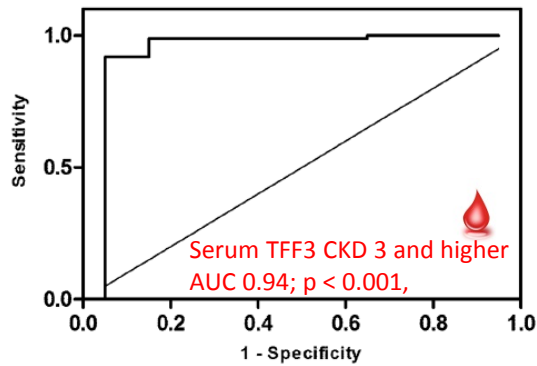
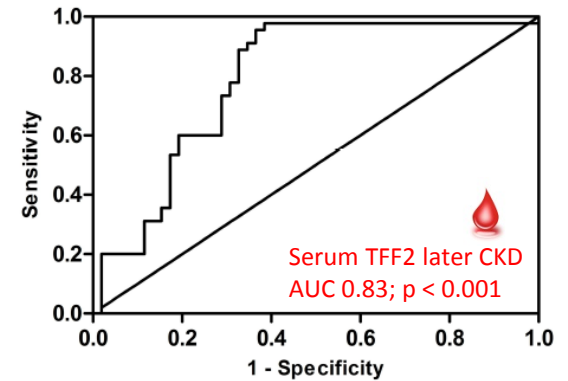
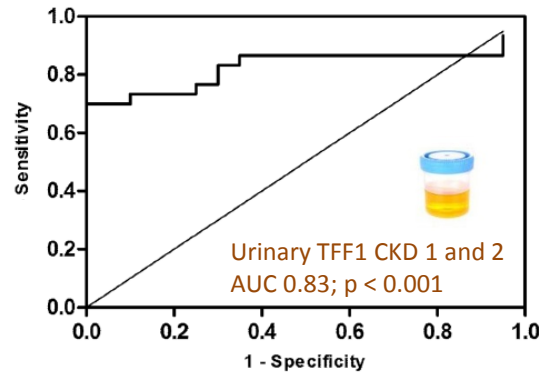
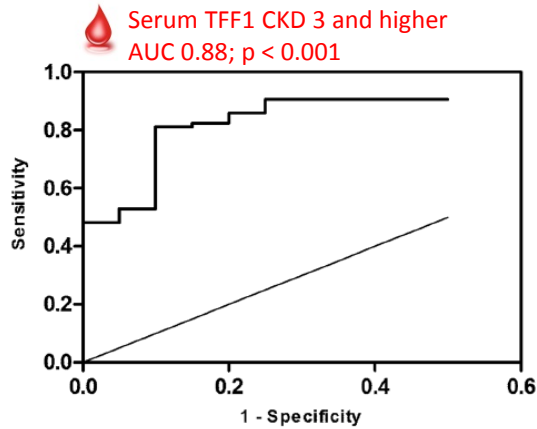
URINE



FRACT. EXCR.



TFF peptides in CKD: Results ROC curve



Chemokines and TFF peptides in Chronic Kidney Disease

- We found elevated fractional chemokine excretion in patients with CKD
- Urinary chemokine levels seem to originate from the diseased kidney itself (sera levels remain unchanged)
- On the other side, chemokine expression can be upregulated during acute diseases irrespective of renal afflictions

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- During the initial phase of kidney disease, TFF1 and the co-regulated TFF2 seems intensively secreted by epithelial renal cells
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 - On the other side, chemokine expression can be upregulated during acute diseases irrespective of renal afflictions
- Initially, TFF1 and TFF2 seem intensively secreted by epithelial renal cells
 - With disease progression, the TFF3 expression seem to be upregulated to additionally limit epithelial cell death and induce restitution.
- the simultaneous evaluation of patients with different causes of CKD might conceal important findings in certain afflictions.
 - Larger clinical studies and longitudinal surveys will be necessary to reveal the role of chemokines and TFF peptides in CKD and during progression to end-stage renal disease

Chemokines and trefoil factor peptides in patients suffering from chronic kidney disease

THANK YOU FOR YOUR ATTENTION

Doctoral thesis at the Medical University of Vienna
for obtaining the academic degree „Doctor of Philosophy“

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Supervisor: Hendrik Jan Ankersmit^{1,3}
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- RAIC Laboratory 13C1²
- Department of Thoracic Surgery³
- Division of Nephrology and Dialysis, Department of Medicine III
- Dept. of Anaesthesia, General Intensive Care and Pain Management⁴