Kidney Blood Press Res 2019;44:200–210

DOI: 10.1159/000498989
Received: October 17, 2018
Accepted: January 3, 2019
Published online: May 8, 2019

Research Article

WNT4 Expression in Primary and Secondary Kidney Diseases: Dependence on Staging

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Keywords
WNT4 · Membranous nephropathy · Lupus nephritis · IgA nephropathy · Focal/segmental glomerulosclerosis

Abstract
Background/Aims: WNT4 protein is important for kidney development. Its expression was found to be altered in experimental models of chronic kidney disease (CKD). However, the expression of the *WNT4* gene has yet not been studied in human renal biopsy samples from patients with broad spectrum of glomerular disease and at different stages of CKD. Thus, the aim of the study was to assess the WNT4 gene expression in renal biopsies of 98 patients using the real-time PCR technique. ***Materials:*** In order to assess the relative amounts of mRNA, in samples of patients with manifestation of different renal diseases and separately at different stages of CKD, by QPCR, total RNA was isolated from human kidney tissues collected during renal biopsies. Results of blood and urine samples assessment were used to calculate the correlations of biochemical parameters with *WNT4* gene expression in both studied groups. ***Results:*** After pathomorphological evaluation, 49 patients were selected as presenting the most common cases in the studied group. Among the patients who developed focal segmental glomerulosclerosis (FSGS; n = 13), IgA nephropathy (IgAN; n = 10), IgAN with morphological presentation of focal segmental glomerulosclerosis (IgAN/FSGS; n = 8), membranous ne-

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phropathy (MN; *n* = 12), and lupus nephritis (LN; *n* = 6) were included in the analysis. We found that the level of *WNT4* mRNA was higher in kidney specimens obtained from patients with MN as compared to those diagnosed with LN or IgAN. A correlation between *WNT4* gene expression and serum albumin and cholesterol levels was observed in patients with FSGS, while *WNT4* mRNA levels correlated with plasma sodium in patients diagnosed with LN. After consideration of 98 patients, based on the KDIGO classification of CKD, 20 patients were classified as CKD1 stage, 23 as stage 2, 13 as stage 3a, 11 as stage 3b, 13 as stage 4, and 18 as stage 5. *WNT4* gene expression was lower in the CKD patients in stage 2 as compared to CKD 3a. Correlations of *WNT4* mRNA level at different stages of CKD with indices of kidney function and lipid metabolism such as serum levels of HDL and LDL cholesterol, TG, urea, creatinine, sodium, and potassium were also found. ***Conclusions:*** Our results suggest that altered *WNT4* gene expression in patients with different types of glomerular diseases and patients at different stages of CKD may play a role in kidney tissue disorganization as well as disease development and progression.

Introduction

Glomerular kidney diseases can be divided into primary and secondary, depending on their background and pathophysiology. IgA nephropathy (IgAN), focal/segmental glomerulosclerosis (FSGS), IgAN with morphological presentation of FSGS (IgAN/FSGS) and membranous nephropathy (MN), start within glomeruli and then lesions spread along the renal tubules and into the interstitial compartment. In the secondary glomerulopathies, glomeruli and tubules become affected in the course of systemic diseases, such as lupus or diabetes, and present as lupus nephritis (LN) or diabetic kidney disease.

Both primary and secondary nephropathies can evolve into chronic kidney disease (CKD) which may progress further into end-stage renal disease (ESRD). CKD is a public health problem with a global dimension. It affects approximately 220 million men and 270 million women worldwide, especially in low- and middle-income countries [1]. CKD is associated with an increased risk of cardiovascular events with high morbidity and mortality. It results (among others) from enhanced oxidative stress, a high degree of "non-specific" inflammation, insulin resistance, anaemia and disturbances of mineral metabolism. CKD is a multi-symptomatic syndrome, uniformly characterized by reduced glomerular filtration rate (GFR) and/or proteinuria. Histopathologically, CKD is characterized by glomerular scarring and sclerosis, tubulo-interstitial inflammation, tubular atrophy and interstitial fibrosis [2].

The molecular background of both primary and secondary nephropathies and CKD has not been completely elucidated and understood [3]. Recently, WNT protein-dependent pathways have attracted attention as the potential contributors to kidney disease development.

Among the 19 protein members of the WNT family, the most important role in kidney disease is assigned to WNT4. WNT4 protein is involved in the kidney development particularly in mesenchymal-to-epithelial transition (MET) [4] and tubulogenesis [5]. *WNT4* gene expression is silenced in normal adult kidney and becomes re-activated in certain pathological situations [6]. WNT4 protein contributes to the regulation of cell cycle progression during tissue regeneration after experimental acute kidney injury (AKI) [7] and reconstruction of damaged tubular epithelium after subcapsular engraftment of Wnt4 expressing fibroblasts in mice [6]. WNT4 protein level increases in the urine samples of patients with renal tubular injury before eGFR lowering becomes apparent and is considered a sensitive indicator of such an injury [8].

Bearing in mind the above, the aim of this study was to detect and determine the level of *WNT4* mRNA using renal biopsy samples of patients with a broad spectrum of primary and secondary glomerular diseases and at different stages of CKD.

Material and Methods

Patients

Stable CKD patients (n = 98; mean age: 47.6 ± 1.63 years) were recruited from patients treated in the Department of Nephrology, Hypertension and Internal Medicine, Faculty of Medicine, University of Warmia and Mazury in Olsztyn, Poland. All study participants provided written informed consent for tissue and blood sample donation (local ethics committee approval: 7/2013/V). The study was performed in patients with clinical suspicion of glomerular disease (primary or secondary) that were subjected to kidney biopsy (which was performed based on standard clinical indications, and not for the purpose of this study). Ongoing acute illnesses (i.e. infection, non-infectious inflammation, cardiovascular event) or disease that had occurred within the last 30 days, known active malignancy as well as drug or alcohol abuse, excluded patients from the study. Diagnosis of the kidney disease and its stages were established by means of clinical, laboratory and histopathological evaluation.

Material Collection and Evaluation of Clinical Parameters

Blood and urine samples were collected from the fasting patients in the morning. Blood samples were centrifuged for 10 min at 3,500 rpm. Biochemical parameters measured in serum included: total protein, albumin, lipid profile, glucose, urea, creatinine and electrolytes; urine albumin was also assayed in 24-h urine. The abovementioned parameters were assayed immediately after sample collection. Cobas 6000 multianalyser (Roche Diagnostics, Basel, Switzerland) and Uro-dipcheck 400e (ERBA, Mannheim, Germany) was used for biochemistry measurements. eGFR was calculated using MDRD formula.

Human kidney tissues were collected during renal biopsies. Kidneys were biopsied based on common indications, i.e. the presence of nephrotic syndrome, subnephrotic proteinuria, isolated (otherwise unexplained) haematuria, an unexplained fall in GFR, as well as when renal manifestations occurred in the course of already diagnosed "systemic" disease or when such a disease was strongly suspected (i.e. systemic lupus). Small tissue fragments were immediately snap-frozen in liquid nitrogen and stored at –80 °C until RNA isolation. Tissue sections for routine diagnostic pathomorphological assessment were fixed in 10% neutral-buffered formalin for 12 h, dehydrated in a graded ethanol series and embedded in paraffin for further processing. Pathomorphological diagnosis was based on routine light and immunofluorescence microscopy evaluation. Transmission electron microscopy was performed in selected cases, when indicated by a renal pathologist.

RNA Extraction and RT-PCR

Total RNA was isolated from the kidney tissue samples using Total RNA Prep Plus kit (A&A Biotechnology, Gdansk, Poland) according to the provided protocol. The quality and quantity of isolated RNA was estimated with a NanoDrop 1000 spectrophotometer (Thermo Fisher Scientific, USA). After reverse transcription conducted according to the protocol of HighCapacity cDNA Reverse Transcription Kit (Applied Biosystem, Foster City, USA), we realized that the size of kidney biopsy samples limited the number of genes and cases that could be analysed. Thus, TaqMan PreAmp Master Mix (Applied Biosystems) were used and preamplification reactions were conducted in Verti thermocycler (Applied Biosystems) in a final volume of 50 µL (200 ng of cDNA diluted to the final concentration volume of 12.5 µL,

12.5 µL of 1:200 diluted pool of primers and probes (20× Taqman Gene Expression Assays *WNT4* – Hs01573504_m1; *TBP* – Hs00427620_m1 and *PPIA* – Hs99999904_m1; Applied Biosystems) and 25 µL of TaqMan PreAmp Master Mix (Applied Biosystems). Cycling conditions were as follows: 37 °C for 2 h and 14 cycles of 85 °C for 5 min. The pre-amplified products were diluted in a 1:10 ratio and used as templates for quantitative real-time PCR reaction in the ABI PRISM 7500 Sequence Detection System (Applied Biosystems).

Real-time PCR reactions (20 µL total contained 5 µL of diluted RT product (1:20), 1 µL of TaqMan probe (*WNT4*-Hs01573504_m1; Applied Biosystems), 10.0 µL of TaqMan Universal Master Mix and 4 µL of water) were run in optimized amplification conditions (50 °C for 2 min, 95 °C for 10 min followed by 40 cycles of 95 °C for 15 s, 6 0 °C for 1 min). Random samples were analysed in the absence of AMV reverse transcriptase. Moreover, non-template control samples were included in each run.

The comparative Ct methods was used [9] for relative quantification of gene expression. Each quantified gene had the Ct normalized against average of *TBP* (Hs00427620_m1) and *PPIA* (Hs99999904_m1) (dCt) – constitutively expressed reference genes [10]. The dCt was not subtracted from control group but from an arbitrary value of 20, so that a high 20-dCt value indicated a high gene expression level.

Classification and Statistical Analysis

After pathomorphological evaluation CKD patients were classified (i) depending on the type of glomerulopathy, and (ii) based on KDIGO classification [11].

The following types of glomerulopathies were diagnosed as the most common: FSGS (n = 13), IgAN (n = 10), IgAN/FSGS (n = 8), MN (n = 12), and LN (n = 6). We decided to divide all our IgAN patients into groups due to high prevalence of IgA nephropathy with distinct morphological appearance of FSGS. For remaining morphological diagnoses, the contribution of biopsies with advanced lesions was less prominent and did not allow for such a distinction. For example, most cases of primary FSGS lesions were classified as "early"; the same applies to LN and MN. In turn, almost all cases of crescent/necrotizing GN (n = 4) were classified as "advanced." The most heterogeneous was the group of transplanted kidney biopsies (n = 14). Out of 31 samples, 5 represented BK virus nephropathy, 5 minimal changes disease, 3 amyloidosis, 2 antibody-mediated (humoral) rejection, 2 secondary FSGS, 1 borderline rejection, 1 acute tubular injury, and 2 normal (healthy) transplanted kidney. In seven cases multiple pathologies were considered and leading diagnosis could not be established.

Based on an eGFR value, 20 patients were classified as CKD stage1, 23 as CKD stage 2, 13 as CKD stage 3a, 11 as CKD stage 3b, 13 as CKD stage 4, and 18 as CKD stage 5 (although we believe that the latter group represented a rapidly progressing manifestation of glomerulopathy). Thus, two types of classification were considered for real-time PCR data analysis.

All statistical analyses were conducted using GraphPad PRISM v. 5.0 software (GraphPad Software, Inc., San Diego, CA, USA). Comparisons of *WNT4* gene expression in kidney biopsy samples of patients with manifestation of different renal diseases and separately at different stages of CKD were conducted with one-way ANOVA followed by Kruskal-Wallis post hoc tests. When two categories of patients were compared, a t Student test was used. Correlations of *WNT4* gene expression in both studied groups with blood and urine biochemical parameters were calculated using a Spearman test. Results of *WNT4* gene expression were presented as Mean ± SEM or Mean 20-dCt ± SD. Differences were considered to be statistically significant at p value less than 0.05.

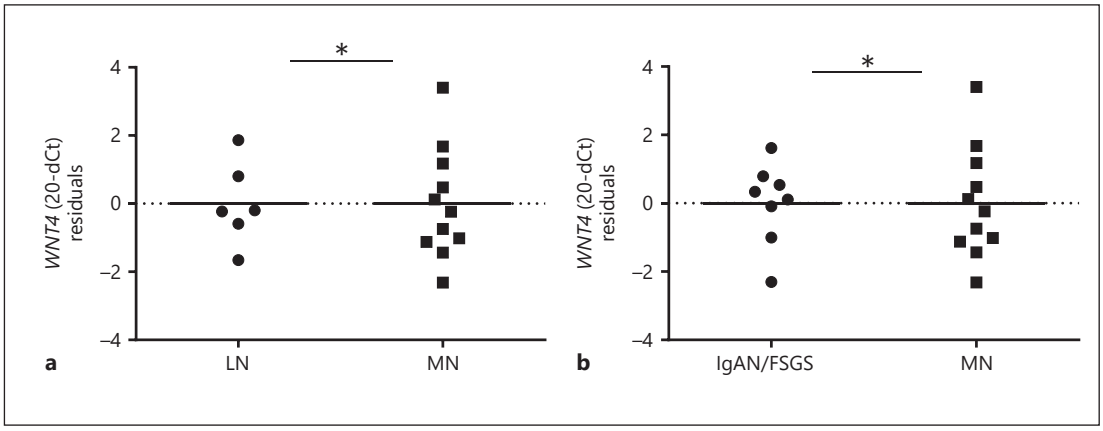

Fig. 1. Quantitative real-time PCR analysis comparing *WNT4* gene expression in kidney biopsies of patients presenting different categories of kidney disease. **a** Comparison of the *WNT4* mRNA level in kidney biopsy samples of patients diagnosed with LN (n = 6) and MN (n = 12). **b** Comparison of the *WNT4* mRNA level in kidney biopsy samples of patients diagnosed with IgAN/FSGS (n = 8) and MN. Gene expression data were normalized against the average of *TBP* and *PPIA* mRNA levels and presented as mean 20-dCt ± SD. Asterisks above the bars indicate statistically significant differences between groups (* $p < 0.05$).

Results

Biochemical Characteristics of Patients

Plasma and urine biochemistry parameters of patients grouped according to different types of glomerular disease and CKD stage are presented in Table 1 and 2, respectively.

WNT4 Gene Expression and Correlations with Clinicopathological Parameters in the Group of Patients with Different Categories of Glomerular Diseases

WNT4 gene expression was influenced by the type of nephropathy. The level of *WNT4* mRNA was higher in biopsies of MN patients compared to those suffering from LN ($p < 0.05$; Fig 1a) and IgAN/FSGS ($p < 0.05$; Fig. 1b).

In patients with IgAN (other than FSGS), IgAN/FSGS and MN, no correlations were found between *WNT4* gene expression and biochemical parameters in plasma and urine (Table 3) with the exception of a negative correlation between *WNT4* gene expression and albuminuria ($r = –0.75$, $p < 0.05$) and cholesterol plasma level ($r = –0.67$, $p < 0.05$) (Table 3) in patients with FSGS. A positive correlation between *WNT4* mRNA level and serum sodium concentration was found in patients diagnosed with LN ($r = 0.94$, $p < 0.05$; Table 3).

WNT4 Gene Expression in the Kidney and Correlations with Clinicopathological Parameters at Different Stages of CKD

Gene expression analysis revealed that the *WNT4* mRNA level did not change in tissue samples across different CKD stages (Fig. 2a). However, the comparison between particular kidney disease groups revealed that *WNT4* gene expression was noticeably lower in CKD stage 2 than in stage 3a ($p < 0.01$; Fig. 2b).

Positive correlations were observed between *WNT4* gene expression and: serum HDL-cholesterol ($r = 0.63$, $p < 0.05$) in patients in stage 3b of CKD, serum TG ($r = 0.63$, $p < 0.05$) in patients with CKD stage 4 and serum sodium ($r = 0.59$, $p < 0.01$) in patients with CKD stage 2. In turn, inverse correlations were found between *WNT4* mRNA and serum LDL-cholesterol ($r = –0.66$, $p < 0.05$) in patients with CKD stage 3a, serum urea (r = –0.61, $p < 0.05$) in patients

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Kidney Blood Press Res 2019;44:200–210
DOI: 10.1159/000498989
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Table 1. Plasma and urine biochemistry results with reference to type of nephropathy

	Units	Total	FSGS	IgAN	IgA/FSGS	MN	LN
N		98	13	10	8	12	6
Age	years	47.64±1.63	46.92±4.4	42.70±6.01	32.63±2.02	53.92±4.49	35.83±4.36
Gender	M/F	60/40	8/5	8/2	5/3	6/6	1/5
Plasma albumin	g/L	32.70±0.99	30.81±2.8	38.43±1.66	38.73±2.70	29.38±2.99	28.13±2.239
Albuminuria	mg/L	685.1±166.1	549.3±86.85	433.7±108.6	444.9±113.3	434.1±153.0	1,397±869.2
Total cholesterol	mg/dL	234.8±8.58	267.4±30.52	202.9±13.62	187.3±16.75	305.5±29.19	265±34.53
HDL	mg/dL	57.73±2.52	56.92±6.58	43.50±5.21	55.50±10.29	69.17±7.98	64.83±5.20
LDL	mg/dL	152.8±7.28	184.9±27.73	132.2±9.42	120.1±13.73	212.6±28.49	166.2±24.79
TG	mg/dL	180.7±8.97	209.6±26.47	155.9±31.46	131.6±31.95	206.5±33.80	227.2±30.50
Glucose	mg/dL	108.2±4.56	92.54±4.14	101.0±6.36	86.50±0.98	102.3±7.13	99.50±19.76
Urea	mg/dL	62.05±3.68	43.54±5.98	45.1±7.80	43.00±4.36	38.92±3.77	33.83±3.28
Creatinine	mg/dL	2.21±0.20	1.27±0.24	1.44±0.24	1.18±0.15	0.95±0.09	0.76±0.07
eGFR	mL/min/1.73 m^2	57.18±3.95	76.66±10.59	65.40±9.48	71.23±8.34	72.73±10.02	95.98±10.68
Sodium	mmol/L	140.2±0.32	140.8±0.95	139.1±1.00	140±0.70	140.5±0.69	140.2±1.05
Potassium	mmol/L	4.41±0.05	4.33±0.1	4.40±0.1	4.64±0.16	4.29±0.1	4.247±0.15

Table 2. Plasma and urine biochemistry results with reference to CKD stage

	Units	Total	CKD1	CKD2	CKD3a	CKD3b	CKD4	CKD5
N		98	20	23	13	11	13	18
Age	years	47.64±1.63	39.37±4.34	44.43±2.77	53.54±4.50	55.36±4.38	50.77±3.58	49.29±3.74
Gender	M/F	60/40	10/10	14/9	6/7	10/1	10/3	9/9
Plasma albumin	g/L	32.70±0.99	28.81±2.33	33.40±1.97	35.73±2.28	30.64±2.80	40.05±2.3	30.30±2.11
Albuminuria	mg/L	685.1±166.1	769±237.3	434.6±86.92	428.3±141.70	453.4±232.10	217.8±123.8	NA
Total cholesterol	mg/dL	234.8±8.58	281.8±20.35	252.1±21.67	224.2±14.35	212.5±20.58	202.9±19.90	205.0±15.88
HDL	mg/dL	57.73±2.52	66.80±5.63	49.48±3.24	67.92±8.07	50.73±3.86	61.25±5.64	52.78±8.09
LDL	mg/dL	152.8±7.28	187.5±18.26	170.7±19.46	149.8±9.64	141.4±17.27	120.5±16.42	122.1±9.35
TG	mg/dL	180.7±8.97	161.9±20.13	215.6±20.15	163.8±22.18	173.7±23.57	173.1±32.21	178.1±15.23
Glucose	mg/dL	108.2±4.56	87.55±3.47	96.52±5.26	108.8±6.56	115.7±17.40	104.2±7.17	143.9±17.69
Urea	mg/dL	62.05±3.68	31.35±2.33	37.09±2.00	50.46±3.84	64.09±5.29	95.23±6.42	111.2±8.12
Creatinine	mg/dL	2.21±0.20	0.68±0.03	1.01±0.04	1.21±0.05	2.00±0.07	2.97±0.22	5.74±0.45
eGFR	mL/min	57.18±3.95	116.2±4.37	74.41±2.13	54.61±1.06	35.13±1.26	22.78±1.33	9.70±0.80
Sodium	mmol/L	140.2±0.32	140.2±0.64	140.6±0.62	140.9±0.93	140.1±0.80	140.7±0.96	139.1±0.91
Potassium	mmol/L	4.41±0.05	4.21±0.08	4.40±0.08	4.29±0.13	4.75±0.14	4.30±0.17	4.60±0.16

NA, not analysed.

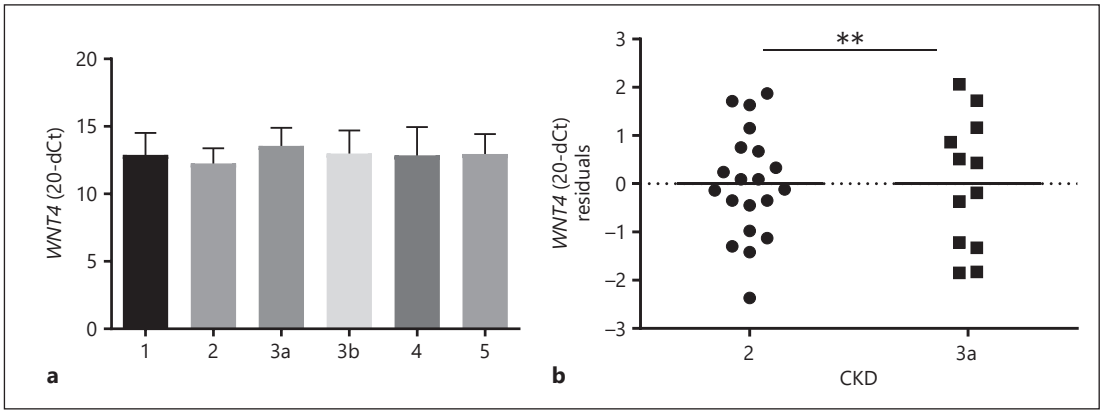

Fig. 2. Quantitative real-time PCR analysis comparing *WNT4* gene expression in the kidney biopsy of patients at different stages of CKD. **a** Comparison of the *WNT4* mRNA level in kidney biopsy samples of 20 patients classified as CKD1, 23 as CKD2, 13 as CKD3a, 11 as CKD3b, 13 as CKD4, and 18 as CKD5. **b** Statistically important difference of the *WNT4* mRNA level in the kidney biopsy samples of patients at 2 and 3a stage of CKD. Gene expression data were normalized against average of *TBP* and *PPIA* and presented as mean 20-dCt ± SD. Asterisks above the bars indicate differences between groups (** $p < 0.01$).

with CKD stage 3 (in both 3a and 3b), serum creatinine in subjects with CKD 3b stage as well as serum sodium ($r = 0.59$, $p < 0.01$) and potassium ($r = –0.62$, $p < 0.05$) in patients with CKD stages 2 and CKD3b, respectively (Table 4).

Discussion

The scarce knowledge concerning the role of the WNT family proteins in kidney disease has been extended by our study which demonstrated *WNT4* gene expression in the kidney biopsy specimens sampled from patients presenting different glomerular kidney diseases, diagnosed based on kidney biopsy and at different stages of CKD. At present, our study seems to be the first in humans since, to date, most of the studies on *WNT4* gene and protein expression have been performed in rodents [5, 6, 8, 12] or comprised very small groups of patients [13]. To the best of our knowledge, no other reports have presented data on *WNT4* gene expression in kidney biopsies of patients with different types of glomerulopathies categorized depending on the type and stage of CKD and correlated with clinicopathological parameters.

Our data show that expression of *WNT4* gene is higher in biopsies of MN patients as compared to those with LN and IgAN/FSGS. Notably, all these glomerulopathies are immune-mediated diseases. Zhao et al. [8] detected the presence of the WNT4 protein in the urine of patients with minimal change disease (MCD) with tubular injury, another disease of an immunological background, and showed that the concentration of WNT4 in urine correlated with histopathological alterations in these patients. In contrast to the clinicopathological characteristics of patients with MCD (normal eGFR and serum creatinine, lack of fibrosis) [8] the kidney biopsies of MN, LN, and IgAN/FSGS of our patients presented glomerular damage and tubulo-interstitial fibrosis. It should be emphasized that in rodent models of MN podocytes become primarily injured through the deposition of immune complexes [14]. However, in experimental models of LN and IgAN/FSGS, podocyte destruction is considered as a secondary event in the process of glomerular injury [14]. The significance of WNT family proteins in

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Kidney Blood Press Res 2019;44:200–210
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Table 3. Correlation coefficients between biochemical parameters of plasma and urine and *WNT4* gene expression in patients with different types of nephropathy

	n	Plasma albumin	Albuminuria	Total cholesterol	HDL	LDL	TG	Glucose	Urea	Creatinine	eGFR	Sodium	Potassium
Units		g/L	mg/L	mg/dL	mg/dL	mg/dL	mg/dL	mg/dL	mg/dL	mg/dL	mL/min/1.73 m^2	mmol/L	mmol/L
FSGS	13	0.14	−0.75*	−0.67*	−0.26	−0.6	−0.5	0.19	−0.10	−0.19	−0.09	0.38	0.24
IgAN	10	0.19	−0.20	−0.28	−0.12	−0.2	0.11	0.30	0.33	0.17	−0.28	0.21	0.47
IgA/FSGS	8	−0.62	0.03	0.48	0.30	0.48	0.52	−0.34	−0.08	−0.40	0.42	0.01	−0.48
MN	12	0.14	0.40	−0.37	−0.16	0.21	0.02	−0.30	−0.22	0.15	−0.05	0.48	0.39
LN	6	−0.54	−0.16	0.03	0.08	0.37	0.43	−0.03	−0.14	−0.2	0.03	0.94*	0.14

Asterisks above the *r* value indicate statistical difference (* $p < 0.5$).

Table 4. Correlation coefficients between biochemical parameters of plasma and urine and *WNT4* gene expression in patients with different stages of CKD according to KDIGO

	n	Plasma albumin	Albuminuria	Total cholesterol	HDL	LDL	TG	Glucose	Urea	Creatinine	eGFR	Sodium	Potassium
Units		g/L	mg/L	mg/dL	mg/dL	mg/dL	mg/dL	mg/dL	mg/dL	mg/dL	mL/min	mmol/L	mmol/L
CKD1–5	98	0.04	−0.07	−0.1	−0.06	−0.09	−0.05	0.12	−0.18	0.07	0.04	0.13	0.006
CKD1	20	0.007	−0.07	−0.16	0.33	−0.17	−0.26	0.17	0.23	−0.17	0.23	−0.36	0.19
CKD2	23	−0.03	0.09	0.06	−0.12	−0.11	0.17	0.11	0.16	−0.22	−0.10	0.59**	0.28
CKD3a	13	−0.02	0.42	−0.16	−0.38	−0.66*	0.15	0.10	−0.61*	−0.23	−0.17	−0.14	−0.06
CKD3b	11	−0.18	−0.60	0.33	0.63*	0.39	−0.05	−0.44	−0.64*	−0.76**	0.45	0.44	−0.62*
CKD4	13	−0.30	NA	0.24	−0.41	0.35	0.63*	−0.16	−0.04	−0.02	−0.24	−0.18	0.21
CKD5	18	0.36	NA	0.16	0.40	0.01	−0.03	−0.14	0.09	0.12	−0.01	0.38	−0.13

Asterisks above the *r* value indicate statistical difference (* $p < 0.5$; ** $p < 0.01$). NA, not analysed.

podocytes was suggested by the work of Maezawa et al. [15] on a conditional Tcf21 knockout mice model. They showed that the expression of WNT4 in podocyte progenitors, in animals with lost activation of Tcf21 transcription factor in developing and mature podocytes, causes the arrest of podocyte differentiation and renal failure in 100% of mice during the perinatal period. In human podocytes from patients diagnosed with FSGS, the immunoexpression of another member of WNT family, WNT1 was markedly induced as compared with the control of non-tumour kidney tissue from the patients who had renal cell carcinoma and underwent nephrectomy [13].

Our findings of the higher WNT4 expression in human MN in comparison to LN and IgAN/FSGS may be partially explained on the basis of experimental and clinical study by [16]. In diabetic nephropathy patients the microarray analysis of glomeruli microdissected from kidney biopsies revealed upregulated expression of the *WNT1, 2B, -4, -6, -16* as well as of *DKK3* (a canonical WNT inhibitor) and *Lef1* (transcription factor) genes. Their experimental study on β-catenin knockout mice that were prone to the development of diabetic nephropathy demonstrated the role of Wnt proteins in epithelial-mesenchymal transition and the activation of intracellular pathways in podocytes [16] as well as their contribution to the pathogenesis of podocyte dysfunction and albuminuria [13]. The results of the above-referred studies and our finding of the variability of *WNT4* gene expression in various types of human glomerulopathies suggest that WNT proteins may play a role in the pathogenesis of these diseases. Further clinical and experimental studies would help in the elucidation of the function of WNT proteins in kidney diseases.

Experimental studies in rodents and cell cultures have shown that the importance of WNT4 protein in renal pathology is not limited to podocytes. Overexpression of *Wnt4* increases cell growth in rat mesangial cells cultured in vitro [17] while down-regulation of *Wnt4* gene expression during experimental diabetes significantly promotes mesangial cell apoptosis and supports mesangial cell survival [17]. Continuous increases of Wnt4 mRNA and protein expression were observed in the course of renal interstitial fibrosis induced by unilateral ureter obstruction (UUO) in mice [18]. Based on the data obtained from four murine models of tubulointerstitial fibrosis Surendran et al. [6] showed that *Wnt4* activation was the highest in the interstitial fibroblasts, suggesting a functional role of WNT4 in renal fibrosis. Thus, the data obtained from animal studies support the results of our human molecular study which suggest that the expression of the *WNT4* gene may contribute to the development of renal lesions in the course of glomerular diseases such as MN, LN, and IgAN/FSGS.

Our study provides the first-in-human evidence of the differences in the expression of the *WNT4* gene that depend on the stage of CKD. These observations have to be confirmed on a larger cohort of patients; however, to some extent, our findings may be compared with the results of a study in an animal model of progressive kidney injury [12]. Our results, to some extent, resemble those obtained in their animal model of moderate-degree AKI: in our trial we observed a slight increase of *WNT4* gene expression while patients were progressing form stage 2 to CKD stage 3a, with *WNT4* gene expression stabilization thereafter. In the study of Xiao et al. [12] inhibition of Wnt/β-catenin signaling hindered progression of AKI into CKD, whereas its overexpression accelerated such a progression.

These findings are intriguing because starting from this range of eGFR *WNT4* gene expression correlates with serum biochemical parameters. Our data to some extent confirm the results of experimental studies by Zhao and coworkers [8] and Wei and coworkers [19] presenting WNT4 as a novel biomarker of kidney tubular injury in minimal change disease and hypertensive mice, as they presented increased *WNT4* expression at a time of renal fibrosis appearance and CKD progression.

Our observation of the correlations between *WNT4* gene expression in kidney biopsies of patients at different stages of CKD with parameters as serum sodium and potassium, urea

and creatinine, indicates the connection between renal histopathological lesions and altered glomerular and tubular function. The correlations with LDL, HDL, and TG serum levels point to the association of *WNT4* gene expression with lipid disorders. In accordance with lipid nephrotoxicity hypothesis abnormalities in lipid homeostasis contribute to renal pathophysiology in patients with CKD [20]. Changes in lipoprotein composition and in cholesterol distribution among plasma, tissues and cellular organelle result from inflammatory, oxidative and endoplasmic reticulum stress, endothelial dysfunction and activation of the renin-angiotensin system [20].

Conclusion

In conclusion, our study is the first conducted on the relatively large cohort of patients which shows *WNT4* gene expression in relation to pathological and clinical features of glomerular diseases in kidney biopsies of patients at different stages of CKD. Our observations in human kidney disease confirm the potential importance of the alterations of WNT4 protein expression in CKD which were earlier reported in animal models of acute and progressive kidney injury.

Acknowledgments

The research was financed from statutory grant (61.610.001-300) of School of Medicine, Collegium Medicum, University of Warmia and Mazury, Olsztyn, Poland.

Disclosure Statement

The authors have no conflicts of interest to declare.

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