Int J Clin Exp Med 2017;10(2):2204-2216
www.ijcem.com /ISSN:1940-5901/IJCEM0038617

Original Article

Analysis of underlying genes and functions associated with diabetic kidney disease

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Received August 24, 2016; Accepted November 27, 2016; Epub February 15, 2017; Published February 28, 2017

Abstract: This study aimed to investigate the pathogenic characteristics of diabetic kidney disease (DKD) from gene level. The microarray data of GSE30528 and GSE30529 were downloaded to screen the differentially expressed genes (DEGs): (a) DKD glomeruli *vs.* normal glomeruli; (b) DKD tubuli *vs.* normal tubuli; (c) normal glomeruli *vs.* normal tubuli. Then functional enrichment analysis and interaction network analysis of the DEGs were performed. Co-expression network analysis was carried out to study the module preservation. Total 511, 503 and 941 DEGs were identified in groups a, b and c respectively. Functional enrichment analyses found that DEGs in DKD glomeruli were mainly enriched in functions related to actin cytoskeleton; in DKD tubuli were mainly enriched in immune and inflammatory response-associated functions. In the interaction networks constructed by DEGs of DKD glomeruli and DKD tubuli, *FYN*, *FN1*, *RB1* and *CASP3* had higher degrees. Co-expression network analysis revealed 43 modules that did not reach the threshold requirements of preservation in DKD glomeruli. The probes in 43 modules had 44 common probes with differentially expressed probes in DKD glomeruli and DKD tubuli, which were enriched in extracellular matrix-associated terms. In the interaction networks of 43 common probes, *FN1*, *ANXA2*, and *EFNB2* had higher degrees. The present study indicates that the function terms like actin cytoskeleton, immune and inflammatory response and extracellular matrix may play an important role in the pathogenesis of DKD. Genes such as *FN1*, *EFNB2* and *ANXA2* may be used as novel biomarkers in DKD.

Keywords: Diabetic kidney disease, functional enrichment analysis, interaction network, co-expression network

Introduction

Diabetic kidney disease (DKD) is a progressive kidney disease as well as a morbid complication of diabetes, which caused by damage to the capillaries in the glomeruli. Glomerulosclerosis and tubulointerstitial fibrosis are the structural hallmarks of advanced DKD [1]. Approximately 40% of persons with diabetes develop DKD [2, 3]. It affects about 15 to 25% patients with type 1 diabetic and 30 to 40% with type 2 diabetic patients [4, 5]. It is reported that, in the United States, DKD is the most common cause of end-stage renal failure which cause severe morbidity and mortality [6]. Therefore, prevention of DKD is important to improve health outcomes of persons with diabetes.

Over the past few decades, studies have elucidated several genes/proteins that play roles in the development of DKD. For instance, various vasoactive factors, such as angiotensin II, endothelin and nitric oxide have been reported to contribute to the progression of DKD [7]. Additionally, in DKD animal models, there is increased gene expression and protein synthesis of extracellular matrix components, such as fibronectin, laminin and type IV collagen, in isolated glomeruli [8]. Furthermore, a recent study by Woroniecka *et al*. [9] presented the gene-expression changes in DKD samples and selected some differentially expressed pathways, such as Cdc42 signaling, Type 1 diabetes signaling, and vascular endothelial growth factor signaling. Although many researches have devoted to the exploration of the genes and pathways associated with DKD, the molecular mechanisms of DKD still remains not fully understood.

Therefore, the present study downloaded the mRNA microarray data of GSE30528 and GSE30529 (provided by Woroniecka *et al.* [9]) from Gene Expression Omnibus (GEO, http://www.ncbi.nlm.nih.gov/geo/) database to screen the differentially expressed genes (DEGs) between

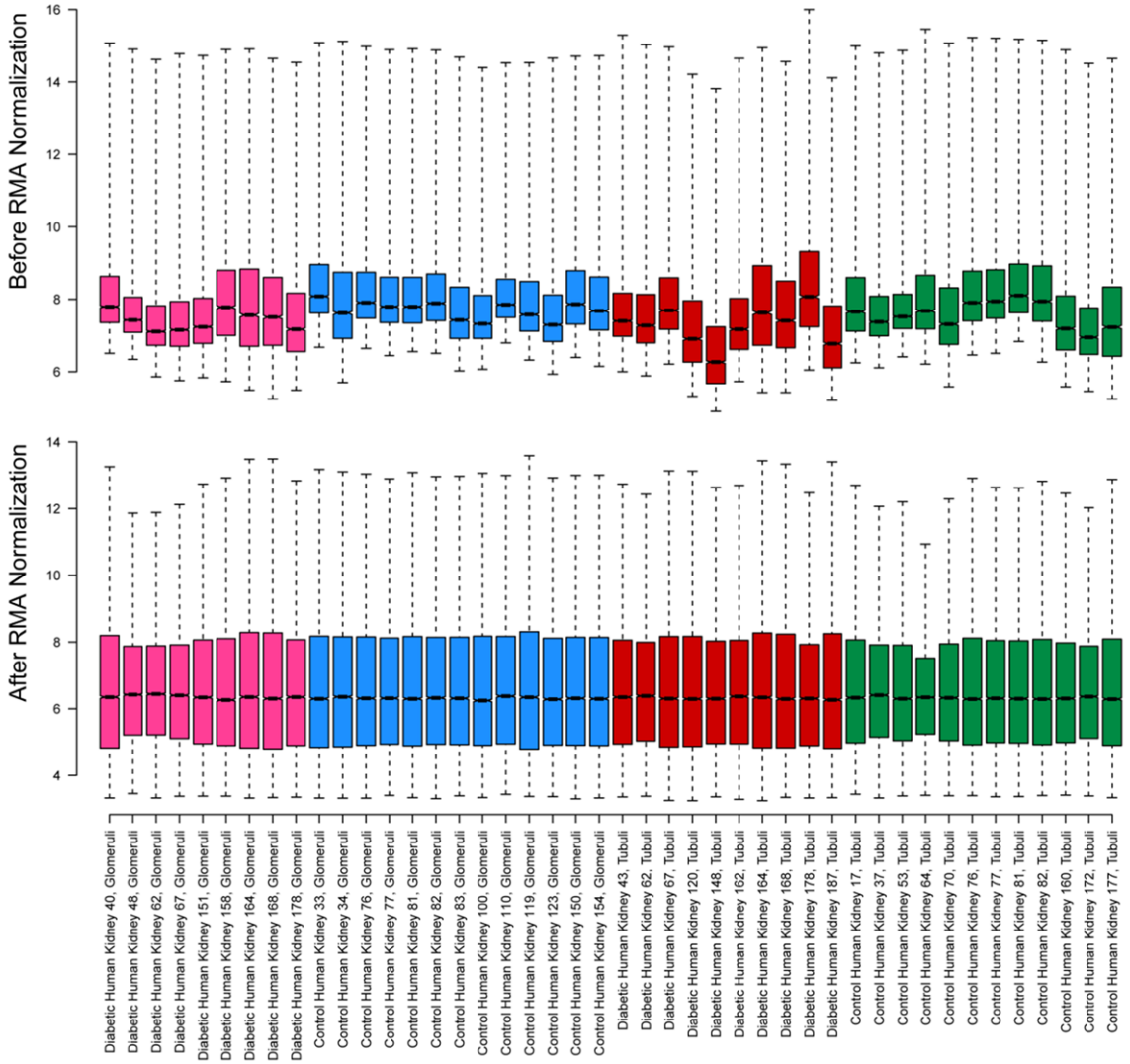

Figure 1. The distribution range of signal value in each sample before and after normalization (n = 44).

DKD glomeruli/tubuli and normal glomeruli/tubuli. Based on the obtained DEGs, comprehensive bioinformatics was applied to analyze the functions and pathways of the DEGs, as well as to construct gene interaction network and co-expression network. This study aimed to investigate the pathogenic characteristic of DKD from gene level and to provide theoretical basis for the diagnosis and treatment of this disease.

Data and methods

Microarray data

Base on the platform of Affymetrix Human Genome U133A 2.0 Array (GPL571 [HG-U133A_2]) (Affymetrix Inc., Santa Clara, California, USA), we downloaded the mRNA microarray data of GSE30528 and GSE30529 from GEO database in the National Center of Biotechnology Information (NCBI). GSE30528 was a glomerulus-associated dataset, including 10 DKD glomeruli and 12 normal glomeruli samples; GSE30529 was a tubuli-associated dataset, including 9 DKD tubuli and 13 normal tubuli samples.

Data preprocessing and principal component analysis (PCA)

The raw data in CEL format were preprocessed using the Affy (version 1.48.0) [10] package in Bioconductor (http://www.bioconductor.org/

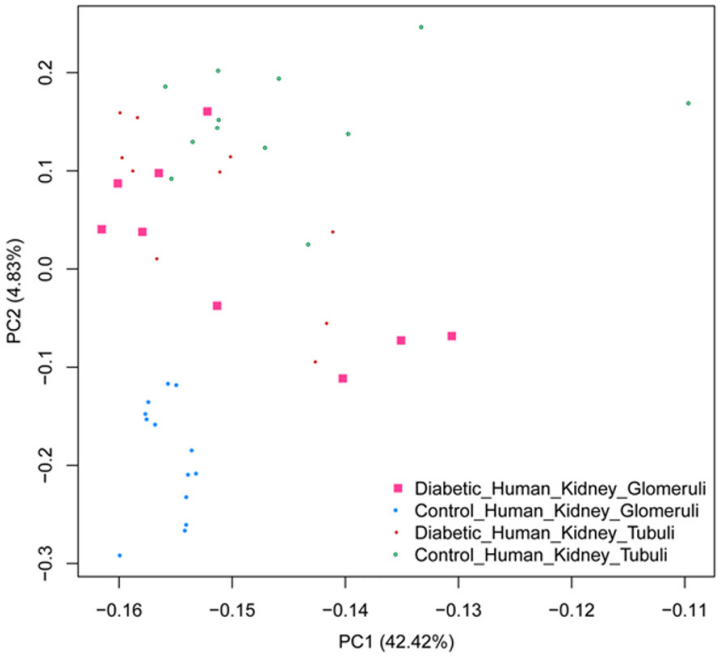

Figure 2. Principal component analysis for all samples (n = 44). 4.83% and 42.42% represent percentage of explained variance.

packages/release/bioc/html/). Then the array data were performed background correction, quartile data normalization and logarithmic transformation using the robust multiarray average (RMA) [11] method in Affy package.

Based on the prcomp function in R software, we performed Principal Component Analysis (PCA) for the expression values of microarray data. Then we reduced the dimensionality of the expression value matrix of all genes to summarize the eigenvector and select the top two principal components that could explain the gene expression difference between samples. If a sample deviated from its groups, it would be removed.

Differential expression analysis

DEGs in two datasets were identified using limma (version 3.26.9, available at http://www.bioconductor.org/packages/release/bioc/html/limma.html) [12] package in R software. The $\log_2$-fold change (logFC) was calculated. *P* value was adjusted by Benjamini & Hochberg (BH) method. |logFC| ≥ 1 and adjusted *p* value ≤ 0.01 were considered as the threshold values for DEGs screening. Considering that there were two datasets, we performed three comparisons: (group a) DKD glomeruli *vs.* normal glomeruli; (group b) DKD tubuli *vs.* normal tubuli; (group c) normal glomeruli *vs.* normal tubuli. Thereinto, normal glomeruli *vs.* normal tubuli was used as a reference of background expression to select the tissue specific DEGs.

Functional enrichment analyses

Database for Annotation, Visualization and Integrated Discovery (DAVID, http://david.abcc.ncifcrf.gov/) [13] is a comprehensive set of functional annotation tool aiming at systematically extracting biological meaning from large gene or protein lists. In order to analyze the DEGs in functional level, we input the DEGs into DAVID (version 6.7) online tool to select the significantly enriched Gene ontology (GO) function and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway. Gene count ≥ 5 and BH adjusted *p* value ≤ 0.01 were set as the threshold values.

Interaction network of DEGs

Based on the Bisogenet plugin (version 3.0.0) in cytoscape (http://www.cytoscape.org/) [14], we searched the known interaction relationships between DGEs, including protein-protein, protein-DNA interactions, from Human Protein Reference Database (HPRD) database. The interaction network was then constructed using the obtained interaction relationships.

Co-expression network analysis

Firstly, we constructed the co-expression network modules for DKD glomeruli, normal glomeruli, DKD tubuli and normal tubuli sample using blockwise Modules function in R package of Whole Genome Co-expression Network Analysis (WGCNA, version 1.51) [15]. Subsequently, we compared the DKD modules with corresponding normal modules to analyze the module preservation and found out the module with significantly changed module preservation. The correlation coefficient of module was

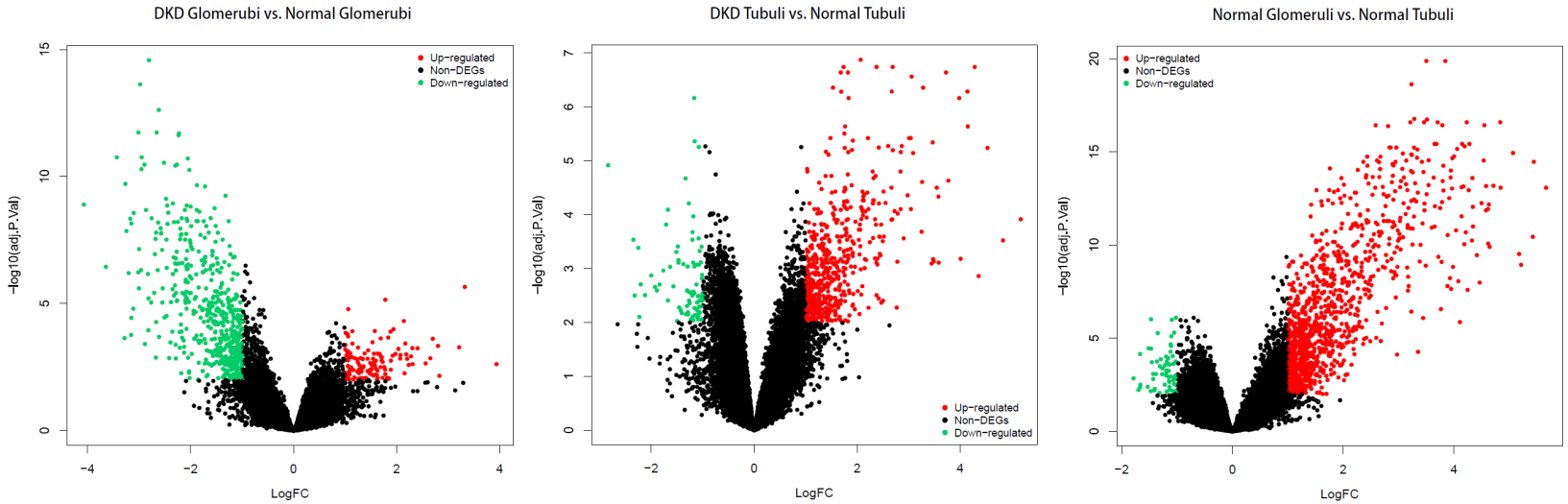

Figure 3. Volcano plots of differentially expressed genes for (A) DKD glomeruli *vs.* normal glomeruli, (B) DKD tubuli *vs.* normal tubuli, (C) normal glomeruli *vs.* normal tubuli. DKD: diabetic kidney disease.

Table 1. The top five significant differentially expressed probes in three groups

ID	Symbol	logFC	*P*.Value	adj.P.Val
DKD glomeruli *vs.* normal glomeruli				
204298_s_at	LOX	-2.80582	1.19E-19	2.65E-15
217452_s_at	B3GALT2	-2.97832	2.13E-18	2.37E-14
37577_at	ARHGAP19	-2.61484	3.25E-17	2.41E-13
210121_at	B3GALT2	-3.01097	3.57E-16	1.86E-12
220889_s_at	CA10	-2.65815	4.18E-16	1.86E-12
DKD tubuli *vs.* normal tubuli				
200665_s_at	SPARC	2.065603	6.01E-12	1.33E-07
211368_s_at	CASP1	2.688225	2.02E-11	1.82E-07
213975_s_at	LYZ	4.281478	3.21E-11	1.82E-07
208690_s_at	PDLIM1	1.73567	4.03E-11	1.82E-07
213293_s_at	TRIM22	2.375053	4.09E-11	1.82E-07
Normal glomeruli *vs.* normal tubuli				
37577_at	ARHGAP19	3.842161	8.72E-25	1.34E-20
204298_s_at	LOX	3.49942	1.20E-24	1.34E-20
212738_at	ARHGAP19	3.232107	3.22E-23	2.38E-19
217452_s_at	B3GALT2	3.281148	3.10E-21	1.72E-17
201930_at	MCM6	3.511662	4.16E-21	1.85E-17

DKD: diabetic kidney disease.

performed pearson correlation analysis using cor.test() function in R, and the statistical data including Z score and *p* value were output. If a DEG had higher co-expression coefficient in both DKD and normal control groups, and its variation direction in the two groups was different, it would be selected for further analysis, including GO enrichment analysis and interaction network analysis.

Results

Data preprocessing

As shown in **Figure 1**, after normalization, the distribution range of each sample microarray was nearly consistent. After PCA for the signal value of each sample, we found that (1) the gene expressions in normal glomeruli and tubuli samples were significantly different; (2) the gene expressions in DKD glomeruli and tubuli samples were similar; (3) the gene expressions in DKD glomeruli and tubuli samples were different from that in normal samples (**Figure 2**).

Differential expression analysis

A total of 642 differentially expressed probes (corresponding to 511 DGEs) were identified in group a, including 122 up-regulated and 520 down-regulated probes (**Figure 3A**). Total 646 differentially expressed probes (corresponding 503 DGEs) were identified in group b, including 562 up-regulated and 84 down-regulated probes (**Figure 3B**). Additionally, 1199 differentially expressed probes were identified in group c, including 1135 up-regulated and 64 down-regulated probes (**Figure 3C**). The top five significant differentially expressed probes in three groups were shown in **Table 1**. Using normal samples as references, the expression levels of most genes in DKD glomeruli were down-regulated while in DKD tubuli were up-regulated.

After comparison of the differentially expressed probes between group a and b, 113 common gene probes were identified (**Figure 4A**), and their correlation coefficient of logFC was 0.67. The expression variation direction of most genes was consistent (**Figure 4B**). In addition, among the 113 common gene probes, 24 probes belonged to group c (**Figure 4A**).

Furthermore, after comparison of the differentially expressed probes between group a and c, and between group b and c, we found that: (1) most of the differentially expressed probes in group a were differentially expressed in group c, while about 40% differentially expressed probes in group b were differentially expressed in group c (**Figure 4A**); (2) most of the 113 common probes were up-regulated in group c (**Figure 5A**), and most of the up-regulated genes in group c were down-regulated in group a and up-regulated in group b (**Figure 5A** and **5B**); (3) most of the up-regulated genes in group c were not significantly differentially expressed in group a and group b (**Figure 5C** and **5D**).

Functional enrichment analyses

After GO and KEGG pathway enrichment analyses, 97 significant terms including 91 GO terms and 6 KEGG pathways were identified in group a, which were related to cell adhesion, and actin cytoskeleton. In group b, 150 significant terms including 138 GO terms and 12 KEGG pathways were identified, which were associated with immune and inflammatory response.

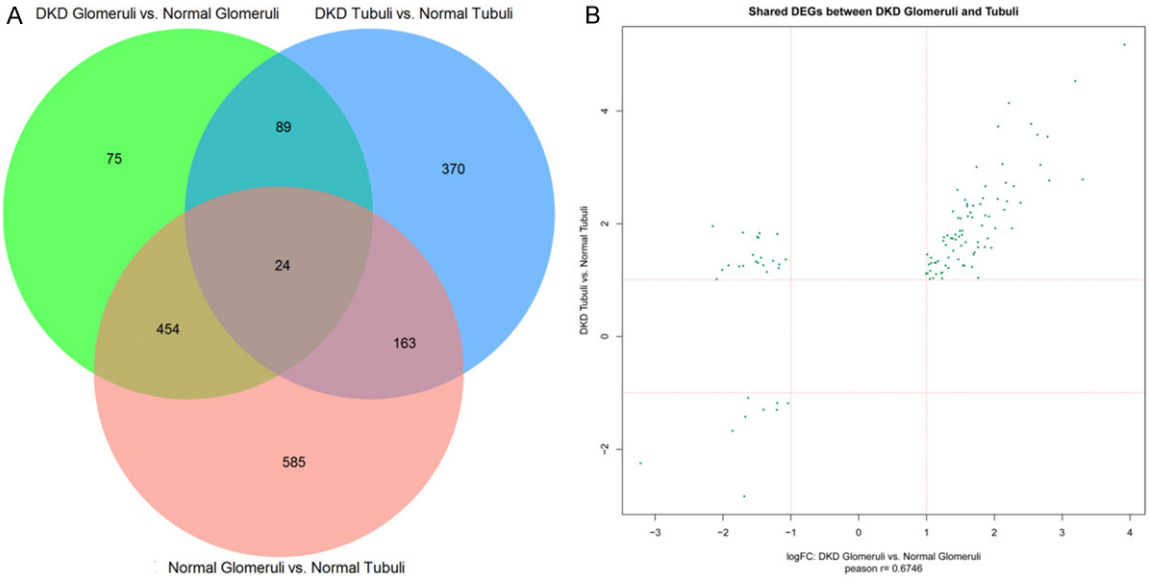

Figure 4. A. Venn diagram of three groups of differentially expressed genes. B. Correlation of the expression variation direction for common genes of DKD glomeruli and DKD tubuli. DKD: diabetic kidney disease.

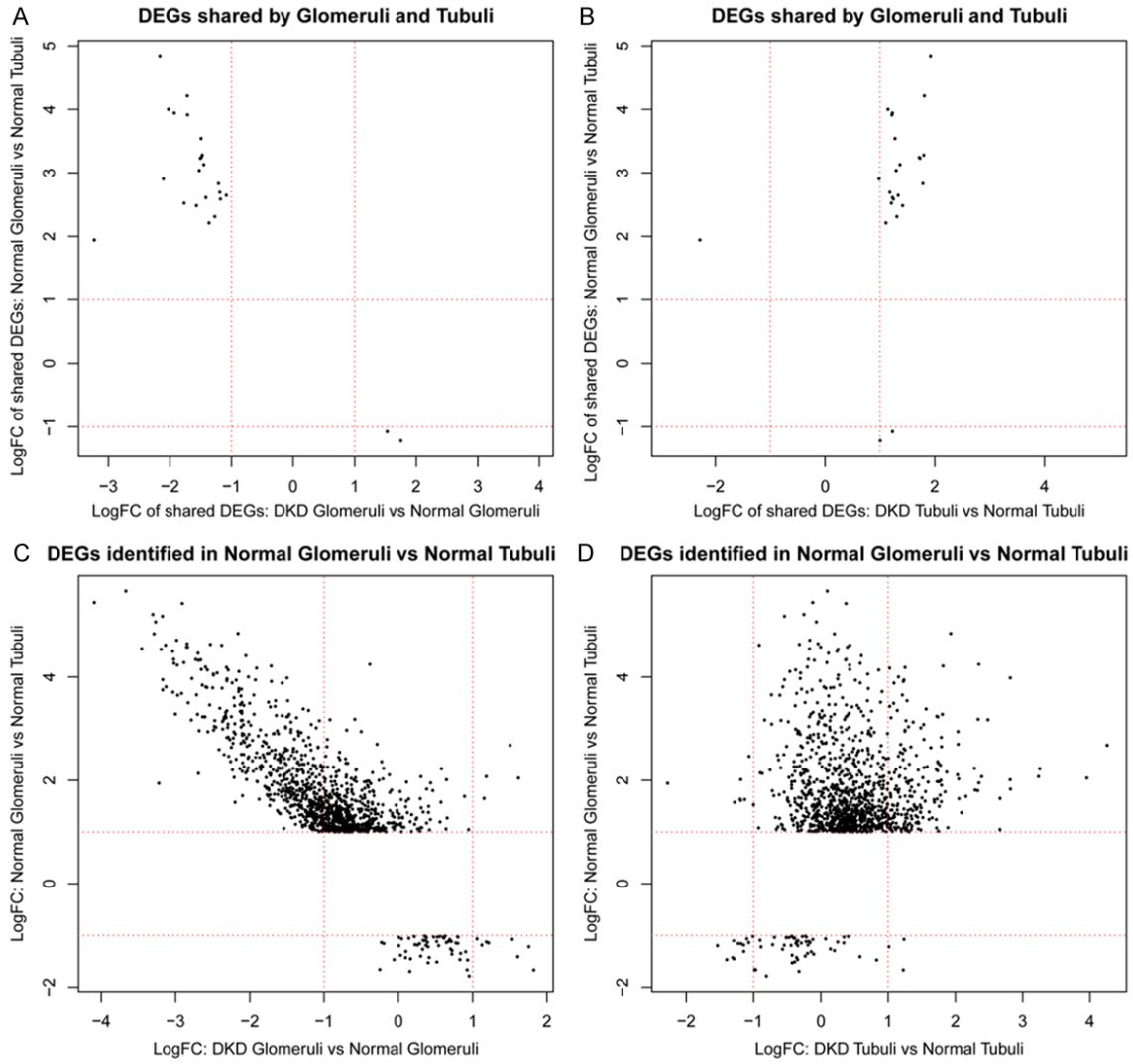

Figure 5. The expression patterns of differentially expressed genes in different groups: A. Fold change relation of differentially expressed genes shared by DKD glomeruli and DKD tubuli in normal and glomeruli groups; B. Fold change relation of differentially expressed genes shared by DKD glomeruli and DKD tubuli in normal and tubuli groups; C. Fold change relation of differentially expressed genes shared by normal glomeruli and normal tubuli in normal and glomeruli groups; D. Fold change relation of differentially expressed genes shared by normal glomeruli and normal tubuli in normal and tubuli groups. Red dotted line represents |logFC| = 1. DKD: diabetic kidney disease.

Table 2. Gene ontology (GO) and kyoto encyclopedia of genes and genomes (KEGG) pathway enrichment analyses for differentially expressed genes in three groups (top three)

Category	Term	Count	Fold enrichment	adj.P.Val
DKD glomeruli *vs.* normal glomeruli				
GOTERM_BP_FAT	GO:0001944~vasculature development	29	3.88805	4.05E-06
GOTERM_BP_FAT	GO:0022610~biological adhesion	51	2.448272	5.48E-06
GOTERM_BP_FAT	GO:0007155~cell adhesion	51	2.45177	8.01E-06
GOTERM_CC_FAT	GO:0015629~actin cytoskeleton	34	4.121349	3.12E-09
GOTERM_CC_FAT	GO:0044459~plasma membrane part	113	1.672541	1.67E-06
GOTERM_CC_FAT	GO:0031091~platelet alpha granule	13	7.569515	1.11E-05
GOTERM_MF_FAT	GO:0008092~cytoskeletal protein binding	49	3.278535	3.57E-10
GOTERM_MF_FAT	GO:0003779~actin binding	35	3.620468	4.81E-08
GOTERM_MF_FAT	GO:0008201~heparin binding	16	5.238381	7.10E-05
KEGG_PATHWAY	hsa04510:Focal adhesion	25	3.418717	1.99E-05
KEGG_PATHWAY	hsa05412:Arrhythmogenic right ventricular cardiomyopathy (ARVC)	12	4.339972	0.004614
KEGG_PATHWAY	hsa04512:ECM-receptor interaction	12	3.926641	0.00581
DKD tubuli *vs.* normal tubuli				
GOTERM_BP_FAT	GO:0006955~immune response	80	3.664635	6.30E-21
GOTERM_BP_FAT	GO:0009611~response to wounding	65	3.876389	7.58E-18
GOTERM_BP_FAT	GO:0006952~defense response	64	3.289233	4.34E-14
GOTERM_CC_FAT	GO:0044421~extracellular region part	81	2.636873	2.73E-13
GOTERM_CC_FAT	GO:0005576~extracellular region	126	1.95907	2.22E-12
GOTERM_CC_FAT	GO:0005615~extracellular space	60	2.737387	2.52E-10
GOTERM_MF_FAT	GO:0030247~polysaccharide binding	26	5.591671	1.65E-09
GOTERM_MF_FAT	GO:0001871~pattern binding	26	5.591671	1.65E-09
GOTERM_MF_FAT	GO:0030246~carbohydrate binding	39	3.648802	1.90E-09
KEGG_PATHWAY	hsa04514:Cell adhesion molecules (CAMs)	21	3.517292	1.01E-04
KEGG_PATHWAY	hsa04512:ECM-receptor interaction	17	4.474379	1.07E-04
KEGG_PATHWAY	hsa05416:Viral myocarditis	14	4.359461	3.20E-04
Normal glomeruli *vs.* normal tubuli				
GOTERM_BP_FAT	GO:0001944~vasculature development	47	3.432427472	8.07E-10
GOTERM_BP_FAT	GO:0001568~blood vessel development	44	3.292030308	1.11E-08
GOTERM_BP_FAT	GO:0022610~biological adhesion	77	2.013492146	4.01E-06
GOTERM_CC_FAT	GO:0044459~plasma membrane part	201	1.658918493	8.06E-12
GOTERM_CC_FAT	GO:0031226~intrinsic to plasma membrane	124	1.855619362	1.66E-09
GOTERM_CC_FAT	GO:0005887~integral to plasma membrane	120	1.836573416	5.23E-09
GOTERM_MF_FAT	GO:0008092~cytoskeletal protein binding	73	2.671128134	1.86E-11
GOTERM_MF_FAT	GO:0003779~actin binding	49	2.771921187	8.67E-08
GOTERM_MF_FAT	GO:0019838~growth factor binding	23	4.039623918	9.70E-06
KEGG_PATHWAY	hsa04514:Cell adhesion molecules (CAMs)	25	2.990894975	1.31E-04
KEGG_PATHWAY	hsa05414:Dilated cardiomyopathy	20	3.433027275	1.53E-04
KEGG_PATHWAY	hsa04810:Regulation of actin cytoskeleton	32	2.350426116	3.52E-04

DKD: diabetic kidney disease; BP: biological process; CC: cellular component; MF: molecular function.

Table 3. 43 not preserved modules identified in diabetic kidney disease glomeruli co-expression network modules

Module color	Module Size	Z score	Log10 *p* value
Orangered	31	1.99070218	-1.927315274
Mediumorchid	64	1.658677241	-1.913746565
Yellow	400	1.494075251	-1.900053313
Orangered3	61	1.824106029	-1.76352924
Skyblue1	62	1.78150353	-1.527281857
Palevioletred3	77	1.782336898	-1.489435478
Pink4	47	1.399125912	-1.426256834
Violet	138	1.673986546	-1.328725094
Lightpink2	38	1.547083222	-1.287377318
Lavenderblush1	38	1.587585477	-1.268873873
Plum	62	1.1645481	-1.204103195
Skyblue3	114	0.988487495	-1.159602914
Coral2	64	1.200331002	-1.158591596
Royalblue	180	1.343710068	-1.11257411
Saddlebrown	154	1.032199802	-1.050095823
Mediumpurple4	50	0.708491959	-0.985786933
Lavenderblush2	52	1.043872463	-0.902814284
Darkseagreen2	36	0.846193751	-0.834059625
Lightpink4	73	0.861663158	-0.825725526
Blue2	58	0.943918277	-0.824386769
Antiquewhite4	66	0.917661914	-0.823070036
Slateblue	33	0.949722642	-0.810047146
Thistle	54	0.91656399	-0.747429564
Pink	400	0.186333414	-0.714768836
Darkolivegreen2	43	0.241499352	-0.69561664
Darkorange2	88	0.440468952	-0.551032817
Salmon2	54	0.417224536	-0.525244312
Tan4	40	0.41697421	-0.502976197
Plum1	110	0.42749128	-0.49800137
Navajowhite	38	0.27414184	-0.455261582
Palevioletred2	54	-0.034733404	-0.452129066
Lightcyan1	100	0.039393674	-0.447864565
Brown4	85	-0.021717606	-0.435467792
Palevioletred1	39	0.219263096	-0.416446212
Green4	37	-0.340958982	-0.377658676
Chocolate4	35	0.001441884	-0.375742202
Sienna4	47	0.01086831	-0.334889062
Salmon1	40	-0.524479671	-0.334667913
Gold	100	-0.11329507	-0.323370546
Antiquewhite1	35	-0.072349642	-0.284554509
Lightsteelblue1	108	-0.618039194	-0.263307405
Lightblue4	45	-0.272677204	-0.256219612
Thistle2	79	-1.292746023	-0.106091145

Additionally, 104 significant terms including 94 GO terms and 10 KEGG pathways were identified in group c, which were associated with vasculature development, and plasma membrane (**Table 2**).

Interaction network construction

From HPRD database, we obtained the interaction relationships of DEGs and constructed the interaction networks. The interaction network for DEGs in group a was shown in Supplementary Figure 1A. In this network, FYN proto-oncogene, Src family tyrosine kinase (*FYN*), LCK proto-oncogene, Src family tyrosine kinase (*LCK*) and fibronectin1 (*FN1*) had the top three highest degrees. Supplementary Figure 1B showed the interaction network for DEGs in group b, and retinoblastoma 1 (*RB1*), caspase 3, apoptosis-related cysteine peptidase (*CASP3*) and vimentin (*VIM*) had the top three highest degrees.

Co-expression network analysis

Using WGCNA, we obtained co-expression network modules of the four sample groups. By comparing DKD network modules with corresponding normal network modules, we found that module preservation of DKD tubuli co-expression network was significant ($P \leq 0.01$), indicating that the gene expression patterns did not change significantly. However, in DKD glomeruli co-expression network modules, 43 modules did not reach the threshold requirements of preservation (**Table 3**).

We extracted all probes of the 43 modules, and extracted intersection probes with the differentially expressed probes in groups a and b, obtaining 44 common probes (corresponding to 33 genes). GO enrichment analysis for the 33 common genes identified 9 significant extracellular matrix-associated GO terms (**Table 4**). In addition, by searching the interaction relationships in HPRD database, we obtained the interaction network for 33 common genes (**Figure 6**). In the network, *FN1*, *LCK*, annexin A2 (*ANXA2*), ephrin-B2 (*EF-*

Table 4. Gene ontology (GO) enrichment analysis for differentially expressed genes identified from the intersection of the probes in 43 modules, differentially expressed probes in DKD glomeruli and DKD tubule

Category	Term	Gene Count	Fold Enrichment	Adjusted *p* value
GOTERM_CC_FAT	GO:0044420~extracellular matrix part	7	24.66887235	2.31E-05
GOTERM_CC_FAT	GO:0005576~extracellular region	17	3.487305408	9.47E-05
GOTERM_BP_FAT	GO:0002526~acute inflammatory response	5	23.00680272	0.021338326
GOTERM_CC_FAT	GO:0005578~proteinaceous extracellular matrix	7	9.019556452	0.002515664
GOTERM_CC_FAT	GO:0031012~extracellular matrix	7	8.365965404	0.002854131
GOTERM_CC_FAT	GO:0005886~plasma membrane	19	2.074167072	0.00902976

DKD: diabetic kidney disease; BP: biological process; CC: cellular component.

NB2) and serpin peptidase inhibitor, clade A (alpha-1 antiproteinase, antitrypsin), member 3 (*SERPINA3*) had higher connectivity degrees, thereinto, *FN1*, *LCK* and *SERPINA3* were up-regulated in both group a and group b. Interestingly, *FN1* and *LCK* also had higher degrees in Supplementary Figure 1A and 1B.

Discussion

In this study, the results showed that hundreds of DEGs were identified in DKD glomeruli and DKD tubuli. Functional enrichment analyses found that DEGs in DKD glomeruli were mainly enriched in functions related to cell adhesion, and actin cytoskeleton; DEGs in DKD tubuli were mainly enriched in immune and inflammatory response-associated functions. In the interaction networks constructed by DEGs of DKD glomeruli and DKD tubuli, *FYN*, *LCK*, *FN1*, *RB1*, *CASP3* and *VIM* had higher degrees. Co-expression network analysis revealed 43 modules in DKD glomeruli did not reach the threshold requirements of preservation. The probes in 43 modules had 44 common probes with differentially expressed probes in DKD glomeruli and DKD tubuli. In the interaction networks of 43 common probes, *FN1*, *LCK*, *ANXA2*, *EFNB2* and *SERPINA3* had higher degrees.

As we known, cell shape are mainly regulated by interconnected actin and microtubule polymers, thus, the cytoskeleton plays an important role in maintaining the normal function of the glomerulus [16]. Previous studies have revealed that cytoskeleton proteins play a very important role in glomerular diseases. Mutations in cytoskeleton genes cause renal disease, leading to rearrangement of the actin cytoskeleton and ultimately to glomerular failure [17-19]. In this study, DEGs in DKD glomeruli were significantly enriched in actin cytoskeleton-related functions, which further suggesting the role of actin cytoskeleton in the function of glomeruli.

DKD is driven primarily by the dysregulation of glucose metabolism pathways, leading to the characteristic loss of blood glucose control which can lead to inflammation and cellular stress [20, 21]. Multiple factors including chronic inflammation have been implicated in DKD tubulointerstitial lesions [22]. In the present study, DEGs in DKD tubuli were mainly enriched in functions associated with immune and inflammatory response. Zheng *et al*. [23] have reported that inflammatory processes are involved in the structural deterioration in DKD. Injured renal cells can release damage signals which trigger remodeling processes by stimulating renal cells and activating immune cells of the innate and adaptive immune systems [22]. Taken together, immune and inflammation may play important roles in the development of DKD.

Co-expression network analysis showed that the gene expression patterns of DKD tubuli were preserved while of DKD glomeruli were not preserved, indicating that the lesions in glomeruli may be the target of DKD treatment. Further analysis identified 44 common probes, such as *FN1*, *EFNB2* and *ANXA2*, which were the intersection of probes in 43 modules that were not preserved in DKD glomeruli, and differentially expressed probes in DKD glomeruli and DKD tubuli. Specially, these gene probes were significantly enriched in extracellular matrix-associated GO terms, which was in accordance with previous findings of Mason et al. [24]. They found that DKD was characterized by

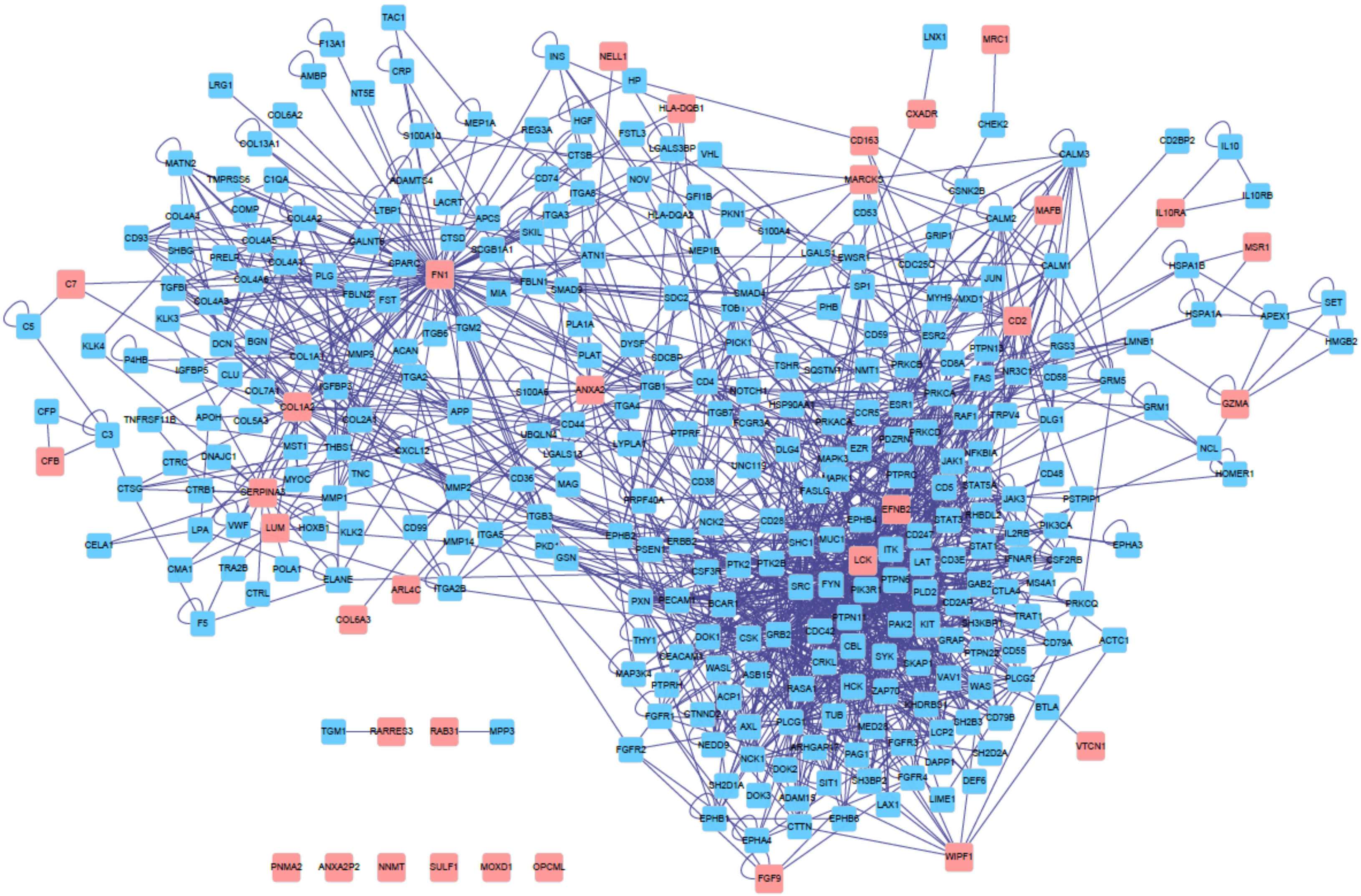

Figure 6. The interaction network constructed by differentially expressed genes identified from the intersection of the probes in 43 modules, differentially expressed probes in DKD glomeruli and DKD tubule. Pink square represents input DEGs, and blue square represents target genes interacted with DEGs. DKD: diabetic kidney disease.

excessive accumulation of extracellular matrix proteins in the mesangium and basement membrane of the glomerulus and in the renal tubulointerstitium.

FN1 encodes fibronectin that is a glycoprotein present at the cell surface and in extracellular matrix, involving in cell-matrix contact processes such as cell adhesion, spreading and migration, and control of cell cytoskeleton and differentiation. It also plays a role in extracellular matrix formation [25-27]. Studies have suggested that impaired interaction with mutant FN leads to disturbance in cell spreading and cytoskeleton in glomerular endothelial cells and podocytes, which is expected to alter the glomerular size-selectivity properties [28, 29]. Castelletti *et al*. [30] demonstrate that mutations in *FN1* are associated with glomerulopathy with fibronectin deposits. Importantly, fibronectin is found to accumulate in excess in DKD. In accordance with the findings above, *FN1* was up-regulated in both DKD glomeruli and DKD tubuli, suggesting that *FN1* may play an important role in the progression of DKD.

EFNB2 encodes a member of the ephrin family which have been implicated in mediating developmental events, especially in the nervous system and erythropoiesis. Previous studies have demonstrated a high level of EFNB2 expression in adult glomeruli [31, 32]. Additionally, EFNB2 is suggested to involve in assembly of glomerular vasculogenesis and maintenance of glomerular structures [33]. Therefore, abnormal expression of *EFNB2* may be associated with DKD. *ANXA2* encodes a member of the annexin family which are a multigene family of Ca^{2+} binding proteins sharing a common property of interacting with membranes in a Ca^{2+}-dependent manner [34]. Annexins are ubiquitously expressed proteins and there is probably no cell type in an organism that would not express a set of annexins [35]. Functional relevance of annexins (including *ANXA2*) expressed in the kidney appears to be linked to several physiological properties important for epithelial cells [35]. We speculated that dysfunction of ANXA2 may be involved in the progression of DKD.

In conclusion, the present study indicates that the GO terms or pathways associated with actin cytoskeleton, immune and inflammatory response, and extracellular matrix may play important roles in the development of DKD. Genes such as *FN1*, *EFNB2* and *ANXA2*may be used as novel biomarkers in DKD. The present results would provide important implications for future research in DKD. However, more experimental studies are still needed to confirm our results.

Acknowledgements

This project was supported by the National Natural Science Foundation of China (No. 81070578 and No. 81270809).

Disclosure of conflict of interest

None.

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