Urinary expression of long non-coding RNA TUG1 in non-diabetic patients with glomerulonephritides

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Abstract. Metabolic alterations serve a significant role in the pathogenesis of kidney disease. Long non-coding RNA (lncRNA) taurine upregulated gene 1 (TUG1) is a known regulator of podocyte health and mitochondrial biogenesis. Although TUG1 protects against podocyte loss in models of diabetic nephropathy, it is unknown if urinary TUG1 expression is associated with clinical and histopathological findings in non-diabetic patients diagnosed with glomerulonephritides. In the present study, the expression of TUG1, podocyte-specific markers (nephrin and podocin) and mitochondrial biogenesis-associated mRNAs (transcription factor A mitochondrial, cytochrome C oxidase subunit 5A and peroxisome proliferator-activated receptor γ coactivator 1α) were examined in urinary sediment of non-diabetic patients with biopsy-confirmed glomerulonephritides and healthy controls. Urinary expression of TUG1 was significantly lower in patients with glomerulonephritides, particularly those diagnosed with Focal Segmental Glomerulosclerosis (FSGS). Furthermore, TUG1 levels were associated with urinary expression of podocyte-specific markers and mRNAs associated with mitochondrial biogenesis. Loss of TUG1 expression in urinary sediment was strongly associated with FSGS, highlighting the potential of this lncRNA and its mitochondrial biogenesis-associated targets as non-invasive biomarkers of assessing podocytopathy.

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Abbreviations: lncRNA, long non-coding RNA; TUG1, taurine upregulated gene 1; TFAM, transcription factor A mitochondrial, COX5A, cytochrome C oxidase subunit 5A; and PPARGC1A, peroxisome proliferator-activated receptor γ coactivator 1α; ESRD, end-stage renal disease; FSGS, focal segmental glomerulosclerosis; DN, diabetic nephropathy; AGS, advanced glomerular sclerosis; NSPH1, nephrin; NSPH2, podocin; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; LN, lupus nephritis; AAV, ANCA-associated vasculitis; GFR, glomerular filtration rate

Key words: TUG1, lncRNA, glomerulonephritides, FSGS, urinary sediment

Introduction

Glomerulonephritides are a group of rare diseases that often affects younger individuals and can lead to end-stage renal disease (ESRD) (1). Although the clinical presentation of glomerulonephritides and their outcomes are variable, well-established factors such as persistent proteinuria, hypertension, diabetes and cardiovascular disease increase the risk of ESRD progression (2). Podocyte injury is a hallmark of renal diseases, presenting clinically with proteinuria, glomerulosclerosis and kidney failure (3). Terminally differentiated podocytes have a limited capacity to self-replicate; hence, direct cellular damage contributes to the onset and progression of glomerular diseases such as Focal Segmental Glomerulosclerosis (FSGS), Minimal Change Disease and Diabetic Nephropathy (DN) (4). Additionally, immune-mediated damage is responsible for the establishment and progression of secondary glomerulonephritides, including lupus nephritis (LN) and ANCA-associated vasculitis (AAV) (5).

The gold standard for confirming a diagnosis of glomerulonephritides diagnosis is renal biopsy (6). However, biopsies are invasive, may have complications and are hard to interpret due to the presence of highly complex pathogenic mechanisms that translate into limited histological responses of kidney injury (7). Thus, identifying non-invasive biomarkers

of glomerular disease may result in improved diagnosis and guide therapeutic choices in the field of nephrology (8).

Long non-coding RNAs (lncRNAs) are RNA molecules comprised of >200 nucleotides with no protein-coding capacity. They are a class of RNAs comprised of heterogeneous intergenic transcripts, sense or antisense transcripts that overlap with other genes, or enhancer RNAs with a range of functions that includes the regulation of gene expression, chromatin remodelling, microRNA (miRNA/miR)-sponging and protein scaffolding (9,10). LncRNAs modulate several biological processes, including homeostasis, cellular metabolism, proliferation, apoptosis and differentiation (10,11). Dysregulated expression of lncRNAs such as metastasis-associated lung adenocarcinoma transcript 1, LOC105374325, LOC105375913, X-inactive specific transcript and RP11-2B6.2 contribute to the pathogenesis of various kidney diseases (12-17). Moreover, circulating lncRNAs are stable and easily detectable in plasma, serum and urine, characteristics that make them convenient for use diagnostically (18,19).

The lncRNA taurine upregulated gene 1 (TUG1), located on chromosome 22q12, regulates podocyte health and glomerulosclerosis by altering the expression of peroxisome proliferator-activated receptor γ Coactivator 1α (PGC1A) (20-23). PGC1A is a transcriptional coactivator that controls mitochondrial biogenesis and is encoded by the *PPARGC1A* gene in humans (24,25). A decrease in PGC1A expression contributes to the onset of metabolic diseases such as DN, and transgenic expression of *PPARGC1A* in tubular cells protects mice from developing acute and chronic forms of kidney disease (26,27). Long *et al* (28) found that podocyte-specific transgenic expression of lncRNA TUG1 protects against DN in a mouse model, and demonstrated that lower glomerular filtration rates (GFR) were correlated with a decrease in TUG1 intrarenal expression in human subjects. However, it is unknown if TUG1 can be detected in urine and if its expression levels are correlated with histopathological findings in kidney biopsies from patients diagnosed with glomerulonephritides other than DN. In the present study, the urinary expression of TUG1 in non-diabetic patients with glomerulonephritides was characterized and it was shown that a decrease in TUG1 expression was significantly associated with FSGS.

Materials and methods

Ethical considerations. The present study complied with the ethical principles for medical research specified in the Declaration of Helsinki (29) and was approved by the Local Ethics and Research Committee at the Hospital de Especialidades NUM. 1, Bajio, Leon, Guanajuato, Instituto Mexicano del Seguro Social (approval no. CLIEIS R-2018-1001-114). Each participant provided written informed consent prior to enrolment in the study.

Patient enrolment. A total of 11 patients with biopsy-confirmed glomerulonephritides (7 females and 4 males; median age, 31 years; range 19-59 years), and 10 healthy controls (6 females and 4 males; median age 34 years; range 22-54) were enrolled in the present study at UMAE-Hospital de Especialidades, CMNO in Guadalajara, Mexico. Inclusion criteria were non-diabetic patients aged ≥18 years. Exclusion criteria were intake of non-steroid anti-inflammatory drugs or platelet anti-aggregation medication a week before sampling, administration of anticoagulant medication 96 h before the biopsy, blood pressure >160/90 mmHg, active urinary infection or anaemia. Urine sample collection was taken shortly before biopsy collection. Only 1 patient enrolled in the study was unable to provide a urine sample and, therefore, was excluded from further analysis.

Data collection. Biochemical parameters corresponding to each glomerulonephritides patient at the time of biopsy were assayed by routine laboratory techniques at UMAE-Hospital de Especialidades and retrieved from patients' electronic medical records.

Reverse transcription-quantitative (RT-q)PCR. Urine specimens from patients and healthy controls were collected and centrifuged at 4,500 x g for 30 min at 4˚C. The urinary sediments were recovered by discarding the supernatant, and total RNA was extracted using RiboZol reagent (Amresco LLC) according to the manufacturer's protocol. cDNA was synthesized by reverse transcription using 1 μg total RNA and a QuantiTect Reverse Transcription kit according to the manufacturer's protocol (Qiagen, Inc.). The cDNA was used to perform quantitative PCR using EvaGreen 5x qPCR mix (qARTA Bio Inc.) and 10 pmol/μl each of the reverse and forward primers in a Lightcycler 96 (Roche Diagnostics). The sequences of the primers used to amplify human lncRNA TUG1 (30), nephrin (NSPH1), podocin (NSPH2), transcription factor A, mitochondrial (TFAM), cytochrome C oxidase subunit 5A (COX5A), PPARGC1A and GAPDH are shown in Table I. The amplification conditions consisted of 1 cycle of initial denaturation at 95˚C for 15 min; followed by 40 cycles of denaturation at 95˚C for 15 sec, annealing at 56˚C for 20 sec and extension at 72˚C for 20 sec. GAPDH was used as the internal control to normalize gene expression data. The relative mRNA expression was calculated using the $2^{-\Delta\Delta Cq}$ method (31).

In silico identification of TUG1/miR-204-5p axis mRNA targets. The VarElect phenotype program from GeneAnalytics (geneanalytics.genecards.org), a pathway enrichment analysis tool, was used to identify predicted mRNA targets of the TUG1/miR-204-5p axis involved in mitochondrial biogenesis based on the genomic information stored in GeneCards (32). In total, 453 predicted mRNA targets of hsa-miR-204-5p downloaded from TargetScan version 7.2 (targetscan.org) (33) were profiled with GeneAnalytics. The entries were scored and ranked using an algorithm that enables matching of genomic expression, sequencing and microarray datasets to tissues and cells.

Statistical analysis. Data are expressed as the mean ± standard error of the mean. Non-parametric variables were compared using a Mann-Whitney U-test or a Kruskal-Wallis test with a Dunn's post-hoc test, as appropriate. A Spearman's rank correlation analysis was used to analyse correlations. Statistical analysis was performed in GraphPad Prism Version 6.01 (GraphPad Software, Inc.). $P<0.05$ was considered to indicate a statistically significant difference.

Results

Clinical and biochemical characteristics of glomerulonephritides patients and healthy controls. The present study included

Table I. Sequences of the primers.

Gene	Sequence, 5'-3'	Size, bp
TUG1		150
Forward	TAGCAGTTCCCCAATCCTTG	
Reverse	CACAAATTCCCATCATTCCC	
NPHS1		124
Forward	GTGCACTATGCTCCCACCAT	
Reverse	TCTCCCAGTTGAACATGCCC	
NPHS2		160
Forward	GAGGAAGGTACCAAATCCTCCG	
Reverse	GCAGATGTCCCAGTCGGAATA	
TFAM		105
Forward	ATGGAGGCAGGAGTTTCGTT	
Reverse	CCTAGATGAGTTCTGCCTGCT	
COX5A		160
Forward	AGATGCCTGGGAATTGCGTA	
Reverse	AGGTCCTGCTTTGTCCTTAACA	
PPARGC1A		158
Forward	TGTGGAGTCCCTGGAATGGA	
Reverse	AAGATCGTGTTGGGCGAGAG	
GAPDH		153
Forward	CCCACTCCTCCACCTTTGAC	
Reverse	TGGTCCAGGGGTCTTACTCC	

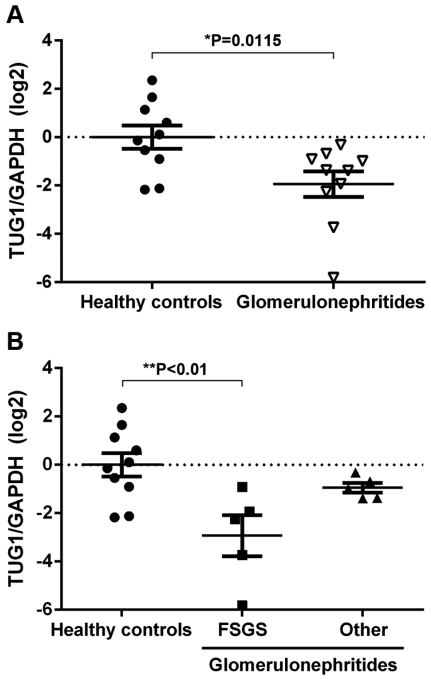

Figure 1. Urinary expression of TUG1 is decreased in FSGS patients. (A) RT-qPCR results showing the $\log_2$-fold change in expression of TUG1/GAPDH in the urinary sediment of healthy controls (n=10) and glomerulonephritides patients (n=10). (B) Reverse transcription-quantitative PCR results showing the $\log_2$-fold change in expression of TUG1/GAPDH in the urinary sediment of healthy controls (n=10), FSGS patients (n=5) and other diagnoses (lupus nephritis, n=3; ANCA-associated vasculitis, n=1; and advanced glomerular sclerosis, n=1). Data are presented as the mean ± standard error of the mean. *P<0.05, **P<0.01. TUG1, taurine upregulated gene 1; FSGS, focal segmental glomerulosclerosis.

10 patients with biopsy-confirmed diagnosis, 4 males and 6 females, and 10 sex-matched healthy controls. The mean age at renal biopsy was 34.4±11.1 years old, the mean body mass index was 30.1±5.1, and the diagnoses were as follows: 5 cases of FSGS, 3 cases of LN, 1 case of AAV and 1 case of advanced glomerular sclerosis (AGS). Table II shows the biochemical parameters of the glomerulonephritides patients. A total of 80% of the patients had haemoglobin values within the normal range. All glomerulonephritides patients had proteinuria, with the highest levels present in the patient diagnosed with AGS. However, CKD staging, classified according to the level of GFR (34) varied greatly. Only 2 patients had a normal GFR of >90 ml/min/1.73 m^2; 3 FSGS patients were Stage 2; 1 AAV patient was Stage 3B; 3 patients were Stage 4 and only 1 LN patient was Stage 5 with a GFR <15 ml/min/1.73m^2. The presence of glomerular damage in the absence of renal failure highlights the importance of identifying novel circulating markers able to detect glomerulonephritides during the early stages of CKD.

Glomerulonephritides patients have low levels of urinary TUG1 expression. Cell-free lncRNAs are stable in urine and may potentially be used as non-invasive biomarkers to identify diseases, such as lupus nephritis and membranous nephropathy (18,19). To assess alterations in TUG1 expression in the urinary sediment of patients with glomerulonephritides, RT-qPCR was used. Urinary expression of TUG1 was reduced in glomerulonephritides patients compared with the healthy controls (Fig. 1A). Furthermore, significantly lower TUG1 expression levels in urinary sediment was observed in patients with FSGS when compared with all other diagnoses (Fig. 1B). These results suggest that the urinary expression of TUG1 decreases with podocytopathy.

Urinary expression of TUG1 correlates with podocyte marker expression. Glomerular injury is associated with podocyte loss, a phenomenon that can be monitored by detecting podocyte-specific mRNAs in the urine (35). In the present study, two podocyte-specific mRNAs, NPHS1 and NPHS2, were detected using RT-qPCR. Although the expression levels of urinary NPHS1 was not significantly different between glomerulonephritides patients and healthy controls, NPHS1 urinary expression was significantly lower in the FSGS patients when compared with other diagnoses (Fig. 2A). In contrast, NPHS2 mRNA did not differ significantly amongst the groups (Fig. 2B). Interestingly, a positive correlation was observed between TUG1 urinary expression and both podocyte-specific mRNAs (NPHS1, r=0.6486, **P<0.01; NPHS2, r=0.4782, *P<0.05; Fig. 2C and D). These results further suggest an association between TUG1 urinary expression and podocyte damage.

Urinary expression of mitochondrial biogenesis markers is correlated with TUG1 expression levels. Several studies have validated the functional role of TUG1 as a lncRNA in directly binding to specific miRNAs to regulate post-transcriptional processing via a competing endogenous RNA mechanism (21,23,28,30). One such target is miR-204-5p (30). Thus, the VarElect phenotype program from Gene Analytics was used to predict mRNA targets of the TUG1/miR-204-5p

Table II. Biochemical parameters of glomerulonephritides patients.

	Patient no.									
Factor	1	2	3	4	5	6	7	8	9	10
Diagnosis	FSGS	FSGS	AAV	LN	AGS	FSGS	LN	LN	FSGS	FSGS
Hemoglobin, g/dl	14.7	13.2	12.8	11.6	8.7	10.5	15.6	13.8	16.7	16.3
sCr, mg/dl	0.84	0.57	1.5	3.7	3.9	2.1	0.86	8.4	1.4	1.5
GFR, ml/min/1.73m^2	90	126	38	17	15	28	107	8	66	63
Proteinuria, g/24 h	1.28	2.68	1.9	2.24	5.8	3.68	1.59	2.9	2.72	1.79
Albumin, g/dl	3.7	2.1	4.1	3.6	2.5	2.3	2.9	3	3.5	4.3
pANCA	+	-	+	+++	-	+	+++	+++	-	-
cANCA	-	-	-	-	-	-	-	-	-	-
Antinuclear antibodies	-	0.09722	0.26389	0.48611	0.09722	0.09722	0.15278	0.15278	0.15278	-
Anti-DNA antibodies	-	-	-	+++	-	-	++	++	-	-
C3 fraction, mg/dl	124	112	93.9	55	104	127	59.7	92.2	143	125
C4 fraction, mg/dl	28.6	38.4	24.4	7	31.8	28.9	9.29	19.4	33.5	33.5
CRP, mg/l	3.75	<3.23	5.53	<3.23	15.8	<3.23	16	11.1	<3.23	<3.23
ESR, mm/h	37	42	16	12	18	-	28	26	-	10

FSGS, focal segmental glomerulosclerosis; LN, lupus nephritis; AVV, ANCA-associated vasculitis; AGS, advanced global sclerosis; sCr, serum creatinine; pANCA, perinuclear/nuclear antineutrophil cytoplasmic antibody; cANCA, cytoplasmic antineutrophil cytoplasmic antibody; C3, complement component 3; C4, complement component 4; CRP, C reactive protein; ESR, erythrocyte sedimentation rate; -, negative; +, low; ++, moderate; +++, high.

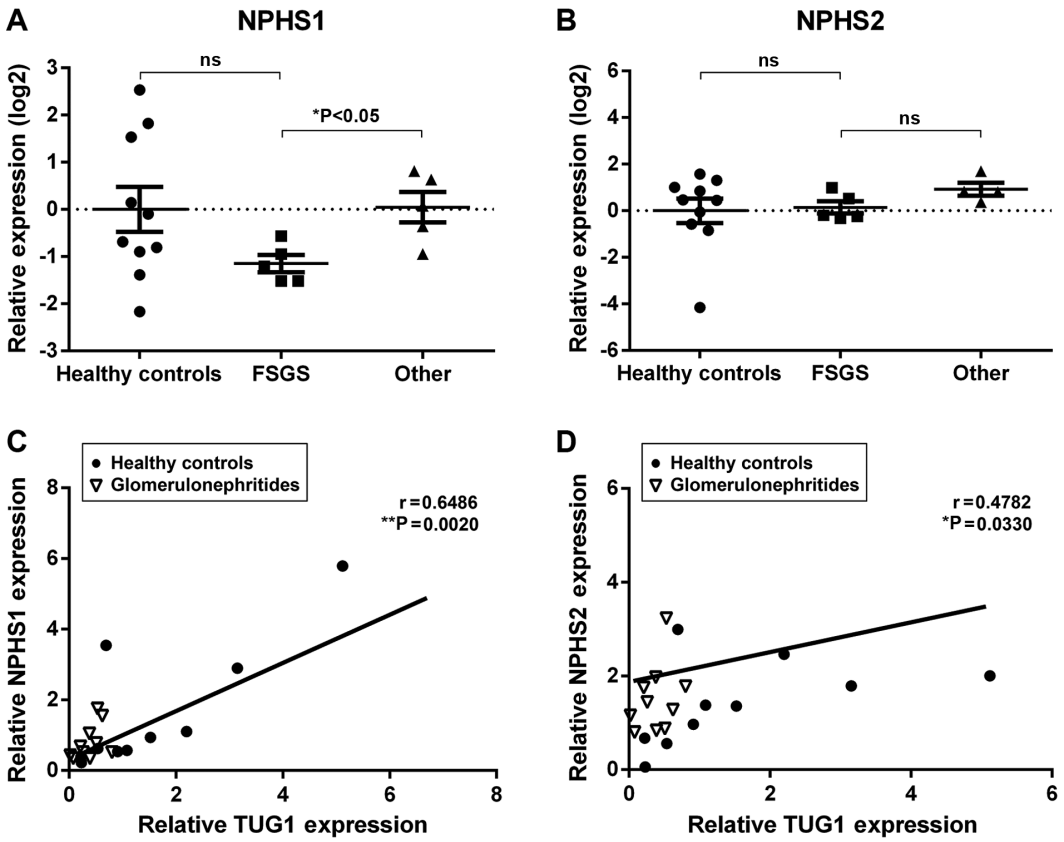

Figure 2. TUG1 urinary expression is correlated with podocyte marker mRNA expression in glomerulonephritides patients and healthy controls. Reverse transcription-quantitative PCR results showing the $\log_2$-fold change in expression of (A) NPHS1 and (B) NPHS2 in the urinary sediment of healthy controls (n=10), FSGS patients (n=5) and other diagnoses (lupus nephritis, n=3; ANCA-associated vasculitis, n=1; and advanced glomerular sclerosis, n=1). Data are presented as the mean ± standard error of the mean. *P<0.05. Correlation between lncRNA TUG1 transcript levels and (C) NPHS1 or (D) NPHS2 in the urinary sediment of healthy controls (n=10) and glomerulonephritides patients (n=10). *P<0.05, **P<0.01. TUG1, taurine upregulated gene 1; NSPH1, nephrin; NSPH2, podocin; ns, not significant.

Table III. Predicted targets of TUG1/miR-204-5p axis involved in mitochondrial biogenesis.

Symbol	Description	Global Rank[a]	Score[a,b]
PPARGC1A	PPARG Coactivator 1α	1	18.76
TFAM	Transcription Factor A, Mitochondrial	4	11.67
CREB1	CAMP Responsive Element Binding Protein 1	25	4.98
SIRT1	Sirtuin 1	26	4.80
COX5A	Cytochrome C Oxidase Subunit 5A	39	3.13
ATF2	Activating Transcription Factor 2	52	2.42
MAPK1	Mitogen-Activated Protein Kinase 1	56	2.32
ESR1	Estrogen Receptor 1	61	1.96
CAMK2D	Calcium/Calmodulin Dependent Protein Kinase II δ	68	1.68
NFATC3	Nuclear Factor of Activated T Cells 3	68	1.68
OGT	O-Linked N-Acetylglucosamine (GlcNAc) Transferase	69	1.59
MXI1	MAX Interactor 1, Dimerization Protein	69	1.59
ESRRG	Estrogen Related Receptor γ	75	0.68
CPOX	Coproporphyrinogen Oxidase	75	0.68
ESR2	Estrogen Receptor 2	80	0.36
TFAP2A	Transcription Factor AP-2α	80	0.36
ZBTB20	Zinc Finger and BTB Domain Containing 20	81	0.26
NPAS3	Neuronal PAS Domain Protein 3	81	0.26
NR4A2	Nuclear Receptor Subfamily 4 Group A Member 2	81	0.26
RICTOR	RPTOR Independent Companion of mTOR Complex 2	81	0.26
CAMK1	Calcium/Calmodulin Dependent Protein Kinase I	81	0.26
TET2	Tet Methylcytosine Dioxygenase 2	81	0.26
ATXN1	Ataxin 1	81	0.26
VHL	Von Hippel-Lindau Tumor Suppressor	81	0.26
HMGCR	3-Hydroxy-3-Methylglutaryl-CoA Reductase	81	0.26
ZNF521	Zinc Finger Protein 521	81	0.26

FSGS, focal segmental glomerulosclerosis; LN, lupus nephritis; AVV, ANCA-associated vasculitis; AGS, advanced global sclerosis; sCr, serum creatinine; CRP, C reactive protein; ESR, erythrocyte sedimentation rate. [a]Obtained from a total of 453 genes. [b]Based on analysis in VarElect of miR-204-5p predicted targets from TargetScan.

axis involved in mitochondrial biogenesis. Through gene data profiling, a total of 27 genes significantly associated with mitochondrial biogenesis were identified (Table III).

Next, the urinary expression of two predicted TUG1/miR-204 mRNA targets significantly associated with mitochondrial biogenesis, TFAM and COX5A, as well as one validated target, PPARGC1A (36), were quantified (Fig. 3). The relative expression levels of COXA5 and PPARGC1A were lower in the urinary sediment of glomerulonephritides patients when compared with healthy controls (Fig. 3C and E). Additionally, there was a significant correlation between TUG1 urinary expression and all three mitochondrial biogenesis mRNAs quantified in the present study (TFAM, r=0.6328, **P<0.01; COXA5, r=0.4872, *P<0.05; PPARGC1A, r=0.7206, **P<0.01; Fig. 3B-F). These results suggest that urinary RNAs may reflect molecular processes involved in the pathophysiology of renal dysfunction.

Discussion

The diagnosis of glomerulonephritides in current clinical practice is primarily dependent on the histological analysis of renal biopsies (6). However, this traditional approach is being challenged by molecular techniques that go beyond description and examine disease mechanisms (37,38). Multiple studies have shown the association between dysregulated expression of certain lncRNAs and the development and progression of pathological disease states that affect the kidney (12-17). Urinary non-coding RNAs are a promising non-invasive tool able to reflect renal disease, aid in its appropriate diagnosis, and guide therapeutic choices (12,39,40). In the present study, it was shown that lncRNA TUG1 was present in the urinary sediment, and its expression was significantly reduced in patients with biopsy-confirmed glomerulonephritides, particularly those diagnosed with FSGS. Moreover, there was a positive correlation between urinary TUG1 expression and mRNAs known to be involved in pathogenic mechanisms associated with podocyte loss.

Mutations in NPHS1 or NPHS2 lead to proteinuria and rapid ESRD progression, highlighting the critical role of these podocyte-specific structural proteins in glomerular filtration barrier function (41,42). Although NPHS1 and NPHS2 are detectable in the urine of glomerulonephritides patients and

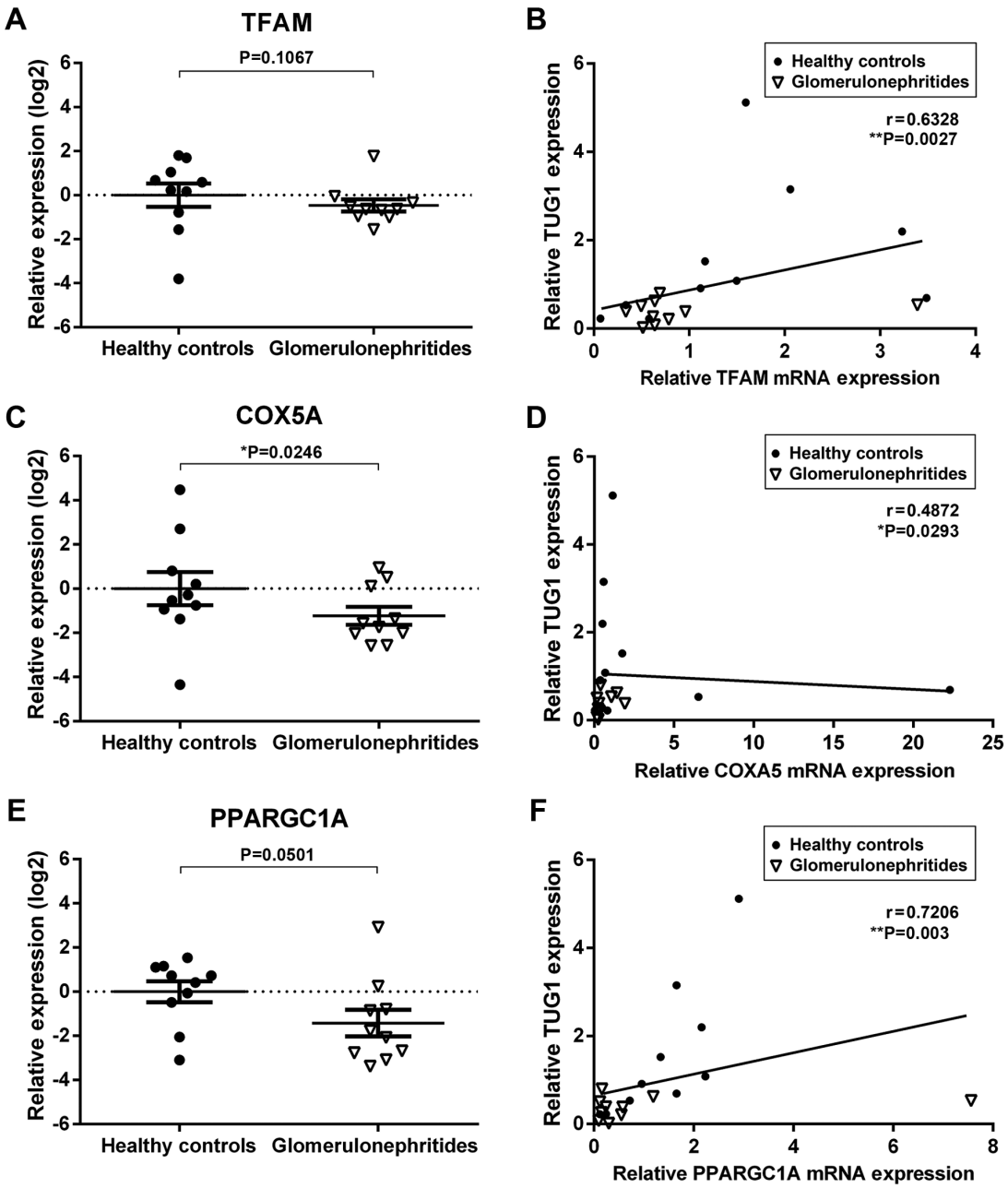

Figure 3. Levels of urinary lncRNA TUG1 correlate with mitochondrial biogenesis mRNAs predicted to be modulated by the TUG1/miR-2014-5p axis. Reverse transcription-quantitative PCR results showing the $\log_2$-fold change in expression of (A) TFAM, (C) COX5A and (E) PPARGC1A in the urinary sediment of healthy controls (n=10) and glomerulonehritides patients (n=10). Data are presented as the mean ± standard error of the mean. Correlation between lncRNA TUG1 transcript levels and (B) TFAM, (D) COXA5 and (F) PARGC1A in the urinary sediment of healthy controls (n=10) and glomerulonephritides patients (n=10). *P<0.05, **P<0.01. TUG1, taurine upregulated gene 1; miR, microRNA; TFAM, transcription factor A mitochondrial, COX5A, cytochrome C oxidase subunit 5A; and PPARGC1A, peroxisome proliferator-activated receptor γ coactivator 1α.

have been described as clinically valuable biomarkers of podocyte injury, the results between studies are inconsistent and raise questions regarding the reasons behind the contrasting findings (43). In the present study, a decrease in urinary NPHS1 expression in FSGS was observed when compared to other diagnoses, but no differences in NPHS2 levels within the groups was found. These results partially agree with previous studies that showed urinary NPHS1 and NPHS2 mRNA levels were reduced in patients with MCN and FSGS, a feature that correlates with the degree of proteinuria (44,45). However, TUG1 expression levels showed no correlation with GFR, proteinuria or albuminuria in the present study (data not shown). Although it has been proposed that these podocyte-specific biomarkers can detect nephropathy before the development of albuminuria (46-48), a larger patient cohort is required to assess the relationship between TUG1 urinary expression and clinical markers of renal dysfunction traditionally used in the diagnosis of glomerulonephritides. In the present study, a positive correlation between NPHS1 and NPHS2 mRNA levels and TUG1 expression was observed, which suggests that the molecular mechanisms described *in vitro* and in animal models of DN regarding the relationship between TUG1 and podocyte loss are reflected in the urine of non-diabetic patients (28).

A decrease in PGC1A, a transcriptional coactivator that regulates energy homeostasis and mitochondrial biogenesis, has been implicated in the development of acute kidney injury, DN and renal fibrosis (49). The expression levels and activity of PGC1A depend on multiple transcriptional and post-transcriptional mechanisms, some mediated by non-coding RNAs (22,28). Interactions between TUG1 and the promoter of PGC1A enhances its transcription, and increases mitochondrial content and cellular ATP levels whilst reducing mitochondrial reactive oxygen species levels. In addition, TUG1 acts as a competitive endogenous RNA for miRNAs such as miR-145, miR-144 and miR-204-5p (30,50,51). miRNA miR-204-5p is involved in multiple cellular processes, including angiogenesis, vascular disease, metabolism and glucose homeostasis (30,52). Through *in silico* analysis, targets of the TUG1/miR-204-5p axis involved in mitochondrial biogenesis were predicted and it was subsequently shown that PGC1A, COX5A and TFAM were also detectable in the urine and were correlated with TUG1 expression. PGC1A is a known posttranscriptional target of miR-204-5p (53,54); however, COX5A and TFAM still require experimental validation. Nonetheless, the results suggest that the TUG1/miR-204 axis and their mitochondrial biogenesis targets are relevant in FSGS pathogenesis and may serve as potential biomarkers.

The present study has some limitations, including the small number of glomerulonephritides patients enrolled and the variability of diagnosis, which included primary and secondary glomerulonephritides. Nonetheless, our findings support a role for lncRNA TUG1 in the development of glomerulonephritides, and justify further exploration of urinary TUG1 as a potential biomarker of FSGS. More extensive studies, with carefully selected subjects are required to confirm the relationship between urinary TUG1 expression, clinical markers of renal dysfunction and glomerular damage observed in kidney biopsies; and to validate lncRNA TUG1 as a biomarker of FSGS. Additionally, more *in vitro* and animal experiments focused on the TUG1/miR-204-5p axis should be performed to increase our understanding of these non-coding RNAs in mitochondrial bioenergetics and podocyte loss.

In conclusion, the results of the present study highlight the potential of urinary non-coding RNAs, such as TUG1, in the diagnosis of renal disease, and suggests that this lncRNA and its mitochondrial-associated pathways are relevant in glomerulonephritides other than DN. Further studies are required to evaluate urinary TUG1 as a potential biomarker of podocytopathy, and to determine its association with kidney dysfunction and patient prognosis.

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Availability of data and materials

The datasets used and/or analysed during the present study are available from the corresponding author on reasonable request.

Authors' contributions

FJS-T, MM-P and RE conceived the study. FJS-T and MM-P collected and analysed the data. ZM performed the experiments. CM-C performed the histopathological analysis of renal biopsies. RE drafted the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The present study complied with the ethical principles for medical research specified in the Declaration of Helsinki and was approved by the Local Ethics and Research Committee at the Hospital de Especialidades NUM. 1, Bajio, Leon, Guanajuato, Instituto Mexicano del Seguro Social (approval no. CLIEIS R-2018-1001-114). Each participant provided written informed consent prior to enrolment in the study.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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