Possible role of Transforming Growth Factor-βeta on podocyte injury and microRNAs regulation in early Fabry nephropathy stages

Transforming Growth Factor-βeta on early Fabry nephropathy

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***Abstract*— Introduction: Fabry nephropathy prognosis worsens when therapeutic interventions are performed in advanced tissue damage stages, since fibrosis and glomerulosclerosis cannot be reversed. Therefore, is of interest, find early kidney damage biomarkers in affected patients. Increased level of urinary podocalyxin (PDCX) reflects specific podocyte damage. Transforming growth factor-β (TGF-β), the main molecule involved in renal fibrosis of any cause, also regulates several miRNAs during renal fibrosis, suggesting that they play essential roles in this process. Fibrosis promoters miRNAs (miR-21, miR-192 and miR-433) are activated by TGF-β, and fibrosis suppressors miRNAs (miR-29 and miR-200 family) are inhibited by TGF-β. The aim of this work is to analyze the association between the expression of urinary TGF-β mRNA with renal fibrosis (urinary miRNA levels) and podocyte injury (urinary PDCX mRNA) biomarkers in Fabry Disease (FD) patients with mild or absent nephropathy. Methods: Diagnostic study. Cross sectional design. Results: 12 FD patients (10.33±3.93 years) and 9 controls (18.66 ± 13.43 years) of similar demographic characteristics were studied. Five patients (41.66%) were TGF-β mRNA sub-expressors and 7 (58.33%) patients were TGF-β mRNA over-expressors. Increased relative excretion levels of urinary TGF-β mRNA in FD patients were associated with statistical significance (p=<0.05; Pearson correlation= -0.683) to increased relative excretion levels of urinary PDCX mRNA. In this group, a urinary excretion of miR-21, miR-29, miR-192, miR-200 and miR-433 similar to controls was observed. In contrast, when compared to control group, "sub-expressor" group (reduced levels of urinary TGF-β mRNA) have presented decreased urinary excretion levels of miR-29 (p=< 0.005) and miR-200 (p=< 0.005), while miR-21, miR-192 and miR-433 remained with levels similar to controls. Conclusions: In early Fabry nephropathy stages, TGF-β could have a dual effect i) being associated with podocyte damage and ii) a probable protective role due to the "normalization" of miRNAs families related to renal fibrosis. FD patients have early podocyte damage compared to healthy subjects. Achieving podocyte protection, both with specific and non-specific therapies, should be an important therapeutic objective during the initial FD kidney damage.**

***Keywords*— Fabry nephropathy; Transforming growth factor-β; microRNAs; Podocalyxin; renal fibrosis**

I. INTRODUCTION

Fabry disease (FD) is an X-linked α-galactosidase-A (αGalA) deficiency which results in glycosphingolipids catabolism failure, particularly globotriaosylceramide (Gb3) and globotriaosylsphingosine (Lyso-Gb3) [1]. This deficiency leads to abnormal multisystemic and progressive storage of unmetabolized substrate [1]. In kidney tissue, Gb3 inclusions have been described in all renal cell types from early age in affected patients. Mainly podocyte inclusions have been directly correlated to age and progression of proteinuria and decrease in estimated glomerular filtration rate (eGFR) [2].
Previous studies have demonstrated podocyte injury in absence of tubulointerstitial fibrosis in young FD patients with mild or absent nephropathy [3]. In addition, a direct correlation between decreased podocytes Gb3 deposits and improvement of albuminuria was observed [4]. These results highlight

the central role of podocytes in Fabry nephropathy early stages [3,4,5].
Pathological albuminuria is the first clinical manifestation of Fabry nephropathy, it can begin in early childhood [1,6] and is produced by glomerular filtration barrier damage, which comprises endothelial cells, glomerular basal membrane (GBM) and podocytes [7]. Microalbuminuria indicates the involvement of these three components in filtration barrier injury [7]. However, proteinuria (or macroalbuminuria) occurs after significant renal damage and does not accurately and sensitively reflect glomerular damage, especially in the early stages [8].
Podocalyxin (PDCX) is a podocyte-associated protein and is expressed on the apical zone of podocyte foot processes [9]. It plays key roles in maintaining the structural and functional integrity of the kidney filtration barrier [9].
PDCX is one of the specific markers for podocyte injury. Due to the proximity of podocyte apical region to the urinary space, it is likely that pathological events occurring in this region are more easily detectable in urine than those occurring in the basal or slit diaphragm regions of podocytes [10].
Increased level of urinary PDCX reflects specific podocyte damage [10] that is unrelated to the other two elements (endothelial cells and GBM), and could be used as a potential non-invasive diagnostic biomarker. Urinary PDCX mRNA correlated with renal parameters, such as microalbuminuria, serum creatinine and eGFR, in the early stages of diabetic nephropathy [12].
Fabry nephropathy prognosis worsens if therapeutic interventions are performed in advanced tissue damage stages, since fibrosis and glomerulosclerosis cannot be reversed [4,13,14]. Therefore, is of interest find early kidney damage biomarkers in affected patients.
MicroRNAs (miRs; miRNAs) are small, endogenous, non-coding RNAs that regulate both physiological and pathological cellular processes. Transforming growth factor-β (TGF-β) regulates several microRNAs during the initial evolution of renal fibrosis. Fibrosis promoters miRNAs (miR-21, miR-192 and miR-433) are activated by TGF-β, and fibrosis suppressors miRNAs (miR-29 and miR-200 family) are inhibited by TGF-β [15-18].
We recently reported a profile of urinary miRNA excretion (miR-21, miR-29, miR-192, miR-200 and miR-433) indicative of renal fibrosis in asymptomatic FD patients with decreased αGalA enzyme activity [19]. In addition, we informed that this probable regulation of urinary miRNAs is not mediated by TGF-β in FD young patients with classical mutations of the GLA gene and mild or absent nephropathy [19].
The aim of this work is to analyze the association between the expression of urinary TGF-β mRNA with renal fibrosis (urinary miRNA levels) and podocyte injury (urinary PDCX mRNA) biomarkers in FD patients with mild or absent nephropathy.

II. METHODS:

A. Participant characteristics

FD patients with confirmed diagnosis by genetic test of any gender, under 18 years of age were included. Exclusion criteria: i) patients with nephropathy by different etiology than FD, ii) patients who at the time of evaluation had any symptoms or intercurrence, and iii) patients with confirmed diagnosis who refused to participate in the study. Elimination criteria: patients with inclusion criteria that presented some complication related to the samples collection process. A population of healthy subjects with similar demographic characteristics was included.
Blood samples and first morning urine were collected from the fasting participants.
All patients had a mutational study by direct sequencing and Multiplex Ligation-dependent Probe Amplification [20,21], and quantification of αGalA enzymatic activity by fluorometric method [22]. Decreased or normal enzyme activity was considered at values less than or > 4.0 nmol/h/l, respectively. Plasma and urine creatinine were determined by electrochemiluminescence (Roche Diagnostics). Albuminuria was determined by colorimetric method (Roche Diagnostics). The urinary albumin/creatinine ratio (uACR) was calculated to estimat 24h albuminuria [23]. Ratio values 0 to 30 were considered normal, 30 to 300 microalbuminuria, and > than 300 macroalbuminuria in at least two samples. eGFR was calculated using Schwartz09 equation (modification 2009) [24].
Neuropathic pain crisis and/or typical acroparesthesias and/or evidence of small neurological fibers damage by Quantitative Sensory Testing (QST) were considered as peripheral nervous system (PNS) symptoms [25,26]. Dyshidrosis and typical GI symptoms were evaluated by questioning and physical examination [27]. Presence of angiokeratomas was evaluated by a dermatologist specialist in FD [1]. Hearing loss was defined by alterations in logoaudiometry test [1]. Presence of cornea verticillata was evaluated by ophthalmological examination with slit lamp [1]. FD cardiac involvement It was defined by: i) cardiac fibrosis: presence of typical images in cardiac magnetic resonance imaging (MRI) with gadolinium and/or ii) cardiac ischemia: presence of typical changes in electrocardiogram and/or cardiac perfusion tests and/or iii) cardiac arrhythmia: presence of electrophysiological disorders in 12-lead electrocardiogram. iv) left ventricular hypertrophy assessed by tissue Doppler echocardiogram and/or cardiac MRI [1,28]. FD central nervous system (CNS) involvement was defined by: i) cerebral white matter lesions in brain MRI angiography and/or clinical stroke by antecedents during the interrogatory, physical examination and demonstration of lesion in cerebral MRI angiography [29].

B. Urine sample preparation and microRNAs extraction

Urine specimen was collected and sent immediately to the laboratory for processing. A volume of 10 ml of urine sample was centrifuged at 3000 x g for 15 min. Nine ml of supernatant was discarded and the remaining milliliter was centrifuged at 15000 x g for 5 min. The urinary cell pellet was stored at -80°C until use. The extraction of microRNAs was performed according to the manufacturer's protocol (NucleoSpin miRNA Plasma kits, Macheney-Nagel, Germany). Currently, there is no available method that can assess the exact quantity or quality of small RNA and standard spectrophotometric methods to measure miRNA performance and quality are not suitable for biological samples. If the yield and concentration of miRNAs are sufficient, the evaluation of the quality of the extraction method can be performed by capillary electrophoresis or reverse transcription (RT) plus real-time polymerase chain reaction (qPCR) [30]. We evaluated the extractions by quantifying the small nucleolar RNA U6 by RT-qPCR. The reaction conditions are described below.

C. microRNAs RT-qPCR

To detect the urinary expression of miR-21, miR-29, miR-192, miR-200 and miR-433 families, RT reaction with a stem-loop primer were used [31]. Stem-loop RT primers were designed according to Chen et al [32]. Primer sequences and the reaction conditions were previously reported by us [18,33].
The specificity of the stem-loop RT primers of each miRNA is given by an extension of six nucleotides at the 3′ end; this extension is inverse and complementary to the last six nucleotides of the 3′ end of the miRNA [33]. MiRNAs were reverse transcribed using Transcriptor First Strand cDNA Synthesis Kit (Roche Diagnostics). Briefly, 5μl total eluate was mixed with 1μM stem-loop RT primer, 0,5μM dNTPs, 1× RT reaction buffer, 20 U RNase inhibitor, 10 U Transcriptor RT and made up to 20 μl with H2O. RT was performed at 16°C for 30min, 42°C for 30 min, 60°C for 60 min and 70°C for 15 min. The resulting cDNA was stored at −80°C until use.
FastStart Universal SYBR Green Master/ROX (Roche Diagnostics) was used for the qPCR reaction, which was performed according to the manufacturer's protocol on a StepOne Plus System (Applied Biosystems). cDNA was amplified using a miRNA-specific forward primer and the universal reverse primer. The forward primers are sequence specific for each miRNA but do not contain the last six nucleotides of the 3′ end of the miRNA. To improve the melting temperature, 5 to 7 nucleotides were added at the 5 'end. RT-qPCR was carried out in compliance with the MIQE guidelines [34]. All qPCR reactions were performed in duplicate, followed by melt curve analysis to verify their specificity and identity. Small nucleolar RNA U6 was selected as the endogenous reference control [35]. Relative miRNA expression levels were calculated using the 2-ΔΔCt method as previously described [36].

D. mRNA extraction and RT-qPCR

Total RNA was extracted according to the manufacturer's protocol (MagNA Pure Compact RNA Isolation Kit, Roche Diagnostics, Switzerland). We confirmed the integrity of RNA by running agarose gel, which was shown to be adequate for PCR. RNA purity was confirmed using the relative absorbance ratio at 260/280 on IMPLEN P330 (Implen, Germany). RNA samples with a ratio higher than 1.8 were used for RT. For reverse transcription, Transcriptor First Strand cDNA Synthesis Kit (Roche Diagnostics, Switzerland) was used. Briefly, 5 µl total eluate was mixed with 2.5 µM anchored-oligo(dT)18 primer and denatured by heating for 10 min at 65°C in a thermal block cycler. Subsequently, it was added 0.5 µM dNTPs, 1x RT reaction buffer, 20 U RNase inhibitor, 10 U Transcriptor RT and made up to 20 µl with H2O. RT was performed at 55ºC for 30 minutes, followed by an inactivation reaction at 85ºC for 5 minutes. The resulting cDNA was stored at -20ºC until use.
In the present study, relative abundance of PDCX and TGF-β mRNA were quantified using the ABI Step One Plus (Applied Biosystems, USA). Human β-actin was used as a reference housekeeping gene. The following oligonucleotide primer sequences were used: TGF-β: forward 5'-CCCTGCCCCTACATTTGGAG, reverse 5'-CCGGGTTATGCTGGTTGTACA; PDCX: forward 5'-CTTGAGACACAGACACAGAG, reverse 5'-CCGTATGCCGCACTTATC; b-actin: forward 5'-TGGCACCCAGCACAATGAA, reverse 5'-CTAAGTCATAGTCCGCCTAGAAGCA.
For qPCR, FastStart Universal SYBR Green Master/ROX (Roche Diagnostics, Switzerland) was used. Briefly, 2 µl cDNA, 1X Master Mix and 150 nM each primers were mixed to make a 20 µl reaction volume. The reactions were incubated at 95°C for 10 min, followed by 40 cycles of 95°C for 15 s and 60°C for 1 min. All qPCR reactions were performed in duplicate, followed by melt curve analysis to verify their specificity and identity. Relative mRNA expression levels were calculated using the ΔΔCt method as previously described [36].

E. Data analysis

Normal distribution of continuous variables was tested using the Shapiro-Wilk Test. Descriptive statistics are presented as means ± standard deviation (SD) or medians and percentiles. For continuous variables, comparison of means/ medians was performed using Student-t-test for variables what followed a normal distribution and Mann-Whitney test/related samples Wilcoxon Signed Rank test for variables who did not. If the qualitative variable had more than two categories, an ANNOVA test was used for variables with normal distribution, and Kruskal-Wallis test was used for those without. For categorical variables, the comparison of the distribution of the variable between

groups was done using the Chi-square or Fisher exact tests. Confidence interval was 95%. Values of p < 0.05 were considered of statistical significance. Data was processed in an IBM SPSS Statistics 20 database (Statistical Package for Social Sciences version 21).

F. Statement on ethics

The study was carried out in accordance with the Declaration of Helsinki for Human Research and approved by the local Ethics Committee. Written informed consent for inclusion was obtained from each participant.

The patients expressed their willingness to participate in the study and their legal representative or guardian, signed the informed consent; according to local legal regulations.

III. Results:

Twenty one participants were included, 12 FD patients (10.33±3.93 years) and 9 controls (18.66 ± 13.43 years).

FD population: 7 females and 5 males of two different genotypes were studied (L415P: 6 patients; E398X: 6 patients). The mean value of eGFR was 152.33±48.39 ml/min/1.73m2 and uACR was 21.75±37.13 mg/g. 1/5 males (20.0%) and 1/7 (14.28%) females had microalbuminuria. No patient had macroalbuminuria. 5/5 males (100%) and 1/7 females (14.28%) had decreased αGalA activity (Table 1).

Control group: healthy subjects (5 women and 4 men) with eGFR and without albuminuria were included (Table 2).

FD patients were classified into two groups according to levels of urinary TGF-β mRNA compared to controls: (i): increased levels of urinary mRNA TGF-β (over-expressors) and (ii): decreased levels of urinary TGF-β mRNA (sub-expressors). In our FD population, there were no patients with equal levels of urinary TGF-β mRNA (normo-expressors) to the controls. Five patients (41.66%) were TGF-β mRNA sub-expressors and 7 (58.33%) patients were TGF-β mRNA over-expressors.

Table 2 shows the variables of the healthy subjects and the FD patients classified in groups. There were no statistically significant differences between the age (p=0.054), eGFR (p=0.192) and uACR (p=0.371) between healthy subjects and FD patients.

When relative excretion levels of urinary TGF-β mRNA were compared between control group and FD patients, we found no statistically significant differences (Figure 1A). However, we observed statistically significant differences in relative excretion levels of urinary PDCX mRNA (p=<0.05) (Figure 1B).

Increased relative excretion levels of urinary TGF-β mRNA in FD patients was associated with statistical significance (p=<0.05; Pearson correlation= -0.683) to increased relative excretion levels of urinary PDCX mRNA (Figure 2).

In the "urinary TGF-β mRNA over-expressors" group, significant difference was associated with decreased αGalA activity (p=<0.001) but not with gender (p=0.235), age (p=0.426) and genotype (p=0.599). In this group, a urinary excretion of miR-21, miR-29, miR-192, miR-200 and miR-433 similar to controls was observed (Figure 3).

In contrast, when compared to control group, "sub-expressor" group (reduced levels of urinary TGF-β mRNA) have presented decreased urinary excretion levels of miR-29 (p=< 0.005) and miR-200 (p=< 0.005), while miR-21, miR-192 and miR-433 remained with levels similar to controls (Figure 3).

IV. Discussion:

In renal injury of different etiology, TGF-β can produce both a reparative and fibrotic response. This molecule is the main factor that drives fibrosis by direct and indirect effects in most forms of chronic kidney disease (CKD), if not all [37]. TGF-β directly acts on renal cells to induce cell proliferation, migration, activation and transcription of profibrotic molecules including collagens, fibronectin, and plasminogen activator inhibitor-1[38,39].

The determination of urinary podocyte and podocyte-derived molecules (proteins and mRNAs) could become a non-invasive tool in the evaluation of glomerular diseases [40]. The majority of urinary podocytes are viable suggesting that they are excreted into urine because of non-apoptotic mechanisms that lead to podocyte dedifferentiation and loss of their specialized characteristics [41]. Podocyte dysfunction occurs during the pathogenesis of glomerular diseases by interference in the expression or deterioration in the structural integrity of any slit diaphragm components [8]. This dysfunction has been studied in several forms of CKD [42]. In animals and experimental models of FD podocytes, the probable rol of TGF-β was described, and a direct correlation between Lyso-Gb3 exposure and the over-expression of TGF-β and extracellular matrix (ECM) components molecules was observed [43,44].

Depending on the magnitude of the lesion, the podocytes are detached from the GBM or undergo structural adjustments to adapt. During the adaptation process, the proteins expressed in the podocytes are released from the cytoskeleton and excreted by urine. The release of urinary podocytes and their expressed proteins seems to be a complex process with multiple participants. The molecular mechanisms of these processes are still not well understood.

Because urine PDCX is markers of podocyte dysfunction detectable in normoalbuminuric CKD patients, this was suggested as an early biomarker whose urinary shedding precedes microalbuminuria, total proteinuria and podocyturia [12,13].

In our study, FD young population had increased urinary PDCX mRNA levels compared to controls. This finding could be interpreted as indicative of glomerular injury, more specifically of podocytes, in early stages of Fabry nephropathy. This result

highlights the central role of podocytes in initial damage on FD renal tissue.

In previous studies, we report that young FD patients with classical mutations of GLA gene and mild or absent nephropathy could present a profile of urinary excretion of miRNAs indicative of renal fibrosis probably not mediated by TGF-ß in FD nephropathy [19,33].

In the current study, our results showed that, sub-expressor group (reduced relative excretion levels of urinary TGF-β mRNA) have decreased relative excretion levels of urinary profibrotic miRNAs (miR-29 and miR-200). In contrast, over-expressor group (increased relative excretion levels of urinary TGF-β mRNA) was associated with i) an increased urinary PDCX mRNA and ii) urinary excretion of miRNAs related to renal fibrosis similar to healthy subjects. This could be interpreted as a probable protective role of TGF-β on these miRNAs families regulation in early stages of FD nephropathy, despite its deleterious effect on podocytes. In this way, miRNAs regulation towards a profile indicative of renal fibrosis could be mediated by pathophysiological pathways different than TGF-β. In this regard, we recently reported an association between high plasmatic Lyso-Gb3 values and decreased relative excretion levels urinary of miRNA with suppressive renal fibrosis effect, miR-29 and miR-200 [45].

In FD, deleterious effects of Gb3 and Lyso-Gb3 on podocytes and renal tubular cells, both mediated by TGF-β, have been reported [43, 44]. Sanchez Niño et al. have described that lyso-Gb3 dose and time dependently increased the TGF-β expression, ECM proteins and CD74, in human Fabry podocytes in vitro [43]. Jeon et al described that FD renal tubular cells treated with Gb3 and Lyso-Gb3 showed a transition from epithelial to mesenchymal cells [44]. A possible deleterious Lyso-Gb3 effect could be a contribution on the miRNAs related to renal fibrosis regulation in FD [45].

In a pilot study on the miRNAs related to renal fibrosis urinary excretion, a probable beneficial effect of enzyme replacement therapy (ERT) with agalsidase beta was described. To date it is the only report that relates an ERT therapeutic effect on urinary miRNAs in FD patients [33].

The small size of the studied population and the cross-sectional design may represent a statistical limitation of the present work. However, other studies have presented similar sample sizes data and methodological design [2,3,4,5,46].

V. Conclusions:

In early Fabry nephropathy stages, TGF-β could have a dual effect i) being associated with podocyte damage and ii) a probable protective role due to the "normalization" of miRNAs families related to renal fibrosis.

FD patients have early podocyte damage compared to healthy subjects. Therefore, it would seem reasonable to consider the early stages of FD nephropathy as a podocytopathy. Achieving podocyte protection, both with specific and non-specific therapies, should be an important therapeutic objective during the initial FD kidney damage.

VI. Abbreviations and Acronyms

αGalA: α-galactosidase-A
CKD: chronic kidney disease
CNS: central nervous system
eGFR: estimated glomerular filtration rate
ECM: extracellular matrix
FD: Fabry disease
Gb3: Globotriaosylceramide
GBM: Glomerular basal membrane
Lyso-Gb: Globotriaosylsphingosine
MRI: magnetic resonance imaging
PNS: peripheral nervous system
PDCX: Podocalyxin
qPCR: real-time polymerase chain reaction
QST: Quantitative Sensory Testing
RT: reverse transcription
SD: standard deviation
TGF-β: Transforming growth factor-β
uACR: urinary albumin/creatinine ratio

VII. Declarations:

A. Ethics approval and consent to participate:

The Ethic Comitee of "Instituto Universitario Italiano de Rosario" approved (Resolution: 26/16) and supervised the study in all its stages. Written informed consent was obtained from each patient or from an appropriate guardian.

B. Consent for publication:

All patients provided necessary consent to participate in the present study. Written informed consent for inclusion was obtained from each participant.

The patients expressed their willingness to participate in the study and their legal representative or guardian, signed the informed consent; according to local legal regulations.

C. Availability of data and material:

The data generated during the study were presented in the manuscript results section.

D. Competing interests:

SJ declares to have received financial contributions from the companies SANOFI-Genzyme, Shire HGT and Biomarin for dissertations on Fabry disease and Mucopolysaccharidosis and financial contributions from SANOFI-GENZYME for research projects; the other authors do not have conflicts of interest related to the contents of this work.

E. Funding:

Not applicable.

F. Authors' contributions:

SJ was involved in the clinical evaluation of patients during the recruitment visit, acquisition, analysis and interpretation of the data and in drafting the manuscript. GV was involved in acquisition, analysis and interpretation of the data and in drafting the manuscript. FP was involved in the clinical evaluation of patients during the recruitment visit and in drafting the manuscript. NA was involved in the clinical evaluation of patients during the recruitment visit and in drafting the manuscript. GP was involved in the completion of laboratory procedures, acquisition, analysis and interpretation of the data and in drafting the manuscript. All authors read and approved the final manuscript.

VIII. FIGURES AND TABLES

Table 1: Clinical and demographic characteristics of Fabry disease patients

ID	Age	Gender	Genotype	αGalA	uACR	eGFR	TGF-β	PDCX
1	5	F	E398X	normal	6.0	138.1	0.18*	-0.[illegible]3*
2	7	M	E398X	decreased	19.0	159.5	-11.0*	-0.36*
3	8	M	E398X	decreased	11.0	174.8	-10.8*	-0.16*
4	12	F	E398X	normal	3.0	179.0	1.19*	0.17*
5	15	F	E398X	normal	39.0	139.7	1.26*	1.97*
6	16	F	E398X	normal	2.0	182.9	-10.6*	-0.82*
7	7	M	L415P	decreased	3.0	128.3	-0.7*	-0.4*
8	7	F	L415P	normal	20.0	162.4	1.08*	1.40*
9	9	F	L415P	normal	6.0	138.3	-10.9*	-0.29*
10	10	M	L415P	decreased	63.0	104.5	-11.2*	-0.58*
11	11	M	L415P	decreased	8.0	185.9	-11.3*	-0.66*
12	17	M	L415P	decreased	135.0	176.0	-11.1*	-0.51*

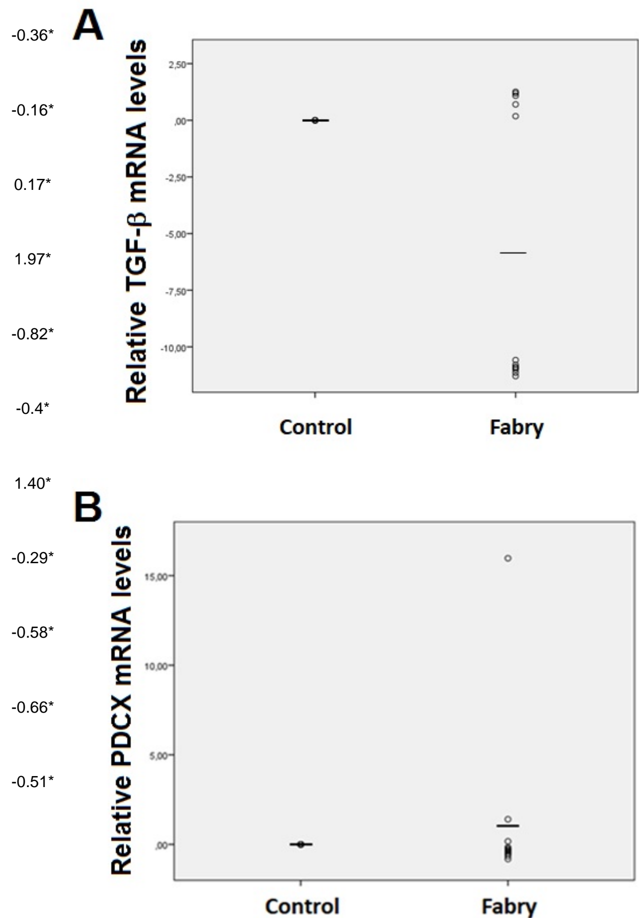
Notes table 1: F: female; M: male; αGal-A: α-galactosidase-A enzyme activity; uACR: urinary albumin/creatinine ratio; eGFR: estimated glomerular filtration rate; TGF-β: urinary Transforming Growth Factor-β mRNA; PDCX: urinary PDCX mRNA. (*) comparitive expression with controls.

Table 2: Variables of controls and Fabry disease patients

	Controls	over-expressors TGF-β patients	sub-expressors TGF-β patients
N	9	6	6
Gender	4M/5F	2M/4F	3M/3F
Age*	18.66±13.43	21.16±26.55	11.14±3.89
eGFR*	121.01±18.9	155.57±23.74	173.75±9.42
uACR*	8.55±5.85	15.50±13.86	30.00±51.76

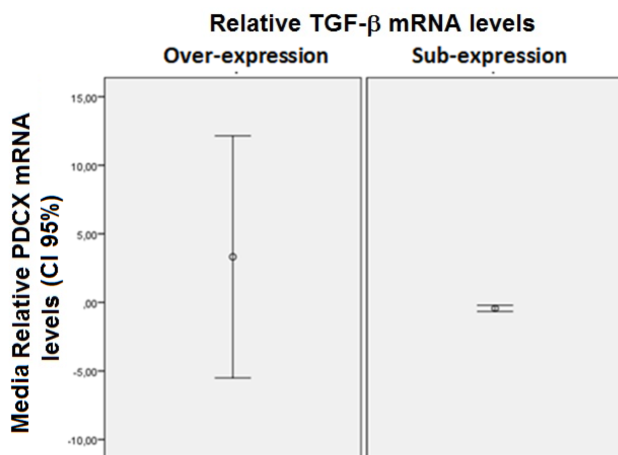
Notes table 2: F: female; M: male; eGFR: estimated glomerular filtration rate; uACR: urinary albumin/creatinine ratio. (*) median ± standard deviation

Figure 1: Urinary TGF-β mRNA (A) and PDCX mRNA (B) comparative expression between Control group and Fabry disease patients.



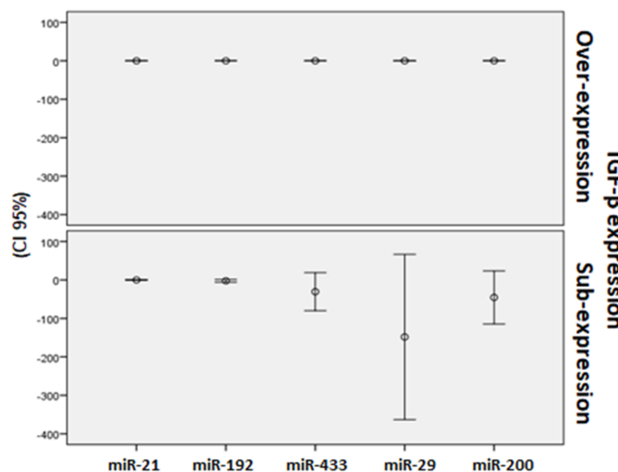
Notes figure 1: Bars represent 2-ΔΔCt values calculated by DeltaDelta Ct (ΔΔCt) method. Expression was normalized to β-actin, and data are represented as means ± SEM.

Figure 2: Correlation between urinary TGF-β mRNA and urinary PDCX mRNA in Fabry disease patients



Notes figure 2: CI: confidence interval; TGF-β: Transforming Growth Factor-βeta; PDCX: podocalyxin.

Figure 3: Correlation between urinary TGF-β mRNA and urinary microRNA excretion in Fabry disease patients



Notes figure 3: CI: confidence interval; TGF-β: Transforming Growth Factor-beta.

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