Evaluation of globotriaosylceramide (GL-3) accumulation in 18 patients with Fabry disease nephropathy. Podocytes are more severely affected than peritubular capillaries.

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ABSTRACT

Fabry disease (FD) is due to deficient α-galactosidase A activity. Kidneys GL-3 accumulation leads to proteinuria, isosthenuria, and progressive failure.

We evaluated baseline GL-3 burden in 9 males and 9 females FD patients who underwent kidney biopsy and were enrolled in three phase 2 studies of migalastat HCl (AT-1001, GR181413A), an investigational pharmacological chaperone. 17/18 patients had a missense mutation. Mean age was 41.5 (18 to 65). 7/18 had proteinuria.

GL-3 was measured in 24-hr urine and homogenized kidney tissue (LC/MS). GL-3 deposition was evaluated semi-quantitatively by histology in podocytes, distal tubular cells and peri-tubular capillaries (PTCs). PTCs were also evaluated with a sensitive quantitative method (BLISS).

16/18 patients had elevated urine GL3 (range 2 to 52 times above normal). Mean GL-3 in homogenized kidney tissue was 3,125 µg/g (range 293-9770). On histology (semi-quantitative), 14/17 patients had the highest podocyte score of '3'. Distal tubular cells, which are more difficult to evaluate, were the second most affected: 5/18 patients were either '2' or '3'. For PTCs, 11/17 biopsies had a score of '0' and 6/17 of '1'. BLISS was more sensitive than semi-quantitative: mean GL-3 inclusions per PTC varied from 0.1 to 5.9. The correlation between PTC inclusions and GL3 tissue content was poor (R^2 0.25), suggesting that most GL-3 accumulated in podocytes and tubular cells.

In FD, GL-3 podocyte accumulation might explain the development of proteinuria and progressive renal failure but it is not a sensitive marker of disease progression. Proteinuria with increased urine GL-3 may indicate podocyte dysfunction. Compared to podocytes, PTCs GL-3 accumulation can be limited.

STUDY DESIGN

•Kidney biopsies were evaluated from 9 males and 9 females from three phase 2 studies of migalastat HCl (AT-1001, GR181413A), an investigational pharmacological chaperone.

•Inclusion criteria were similar for all studies:

- •Male and female subjects between 18 and 65 years of age with a confirmed diagnosis of FD were enrolled.
- •A missense mutation in the GLA gene with residual α-Gal A activity of at least 3% of normal were required, as was the demonstration of enhancement α-Gal A activity by AT-1001 in patients cultured lymphocytes. The initial criteria for enhancement required α-Gal A activity to be a relative increase of at least 20% in the presence of 20 µM AT1001 in the lymphocyte assay (Developmental and Metabolic Neurology Branch, NIH, Bethesda MD and the Royal Free Hospital, London, UK).
- •Patients were to be naïve to ERT or willing to stop ERT for the duration of the study, including treatment extensions.

•Key exclusion criteria were:

- • Significant disease or organ dysfunction
- • a serum creatinine greater than 2 mg/dL
- •QTc interval > 450 ms.

Bioanalytical and Histological Analyses

GL-3 was evaluated using multiple methods:

GL-3 by LC/MS:

GL-3 content was measured in 24-hr urine and homogenized kidney tissue via LC/MS.

Urine GL-3:

Measurement of urine GL-3 was conducted at Department of Genetic Medicine, Women's and Children's Hospital, North Adelaide, Australia. Whole urine was subject to sonication to lyse cells. Urinary lipids from sediment and supernatant were extracted by liquid-liquid extraction. GL-3 was reported as the sum of five isoforms (C16:0, C20:0, C22:0, C24:0, C24:1). The lower limit of detection was approximately 1 ng/mL. To correct for urinary sediment cell content, GL-3 was normalized to total phosphatidylcholine (PC) determined in the same LC-MS/MS assay. Results were expressed in pmol total GL-3/nmol PC [1] and expressed as fold-time above the Upper Limit of Normal (ULN).

Kidney GL-3:

GL-3 was measured in kidney tissue homogenates with LC-MS/MS (MDS Pharma Services, Montreal, Canada). GL-3 was reported as the sum of nine different isoforms. Results were expressed as micrograms GL-3/grams of tissue used in the homogenate.

GL-3 by histology:

GL-3 deposition was evaluated semi-quantitatively by histology in podocytes, distal tubular cells and peri-tubular capillaries (PTCs) [2]. PTCs were also evaluated with a sensitive quantitative method (BLISS) [3].

Semi-quantitative scoring:

GL-3 was evaluated in kidney cells by evaluating GL-3 content by light microscopy (LM). Sections were stained with methylene blue-Azure. A GL-3 score was assigned by a single pathologist based on a semi-quantitative scale of severity, with zero representing no GL-3 and three representing the most severe levels of GL-3 accumulation [2]. Cells included podocytes, distal tubular cells and peritubular capillaries (PTCs).

BLISS scoring:

For kidney PTCs a novel scoring system, the Barisoni Lysosomal Inclusion Scoring System (BLISS) [3], was applied. BLISS-LM has been shown to have improved sensitivity and better reflect GL-3 load compared to a semi-quantitative method [2]. Two pathologists each independently counted GL-3 inclusions per capillary in approx. 60-80 capillaries, and recorded the average score per PTC. The final BLISS score used for analysis was the average of these 2 scores.

Results are reported descriptively. A linear correlation between the log10 of GL-3 tissue content and BLISS-LM results in PTC was performed.

Study identification were the following: FAB-CL-202, NCT00283959 and FAB-CL-203, NCT00283933 FAB-CL-204/NCT 00304512.

- Data presented are pre-treatment (baseline data)

RESULTS

Table 1: Subject demographics, mutation and clinical status #

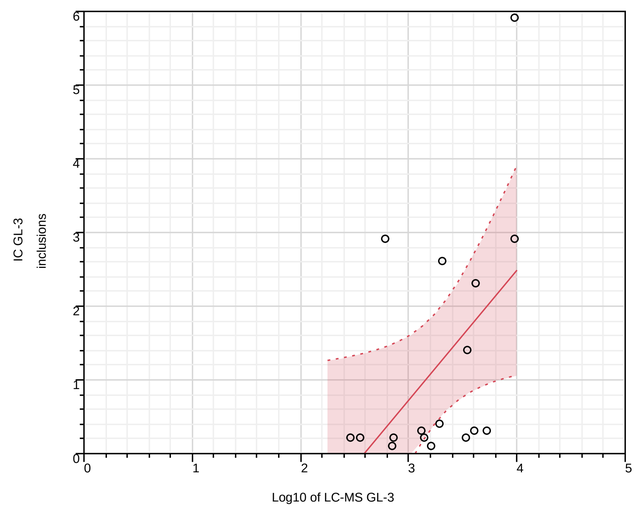
Subject	Sex/age	Amino acid change	Fabry disease clinical status
4 01-01	F/62	*P259R*	Bradycardia, 2d degree atrio-ventricular block, LVH
4 01-02	F/39	*P259R*	Acroparesthesia, bradycardia, proteinuria
4 02-01	F/59	*P205T*	Acroparesthesia, bradycardia, LVH, transient ischemic attack
4 02-03	F/36	*C52G*	Hearing loss, LVH, depression, proteinuria
4 03-01	F/42	*R227X*	Angiokeratoma, acroparesthesia, abdominal pain, depression
4 03-02	F/36	*R112H*	Angiokeratoma, acroparesthesia, abdominal pain, depression, transient ischemic attack
4 03-05	F/43	*L32P*	Acroparesthesia, abdominal pain, depression
4 04-02	F/44	*E358K*	Angiokeratoma, acroparesthesia, bradycardia, proteinuria
4 06-03	F/47	*M1I*	Bradycardia, short PR interval, LVH, proteinuria
2 01-02	M/27	*L415P*	Angiokeratoma, acroparesthesia
2 01-03	M/23	*P259R*	Acroparesthesia, bradycardia, LVH, hearing loss
2 01-04	M/18	*P259R*	Angiokeratoma, bradycardia, short PR interval, diarrhea
2 02-02	M/65	*R301Q*	Angiokeratoma, bradycardia, RBBB, LVH, renal insufficiency, hearing loss, peri-ventricular white matter change, proteinuria
3 03-01	M/39	*F295C*	Angiokeratoma, acroparesthesia, abdominal pain, bradycardia, LVH, proteinuria
3 03-02	M/31	*C94S*	Angiokeratoma, acroparesthesia, bradycardia, hearing loss
3 03-03	M/36	*R112C*	Angiokeratoma, acroparesthesia, hearing loss, renal insufficiency, proteinuria
3 RF-01	M/55	*N215S*	Angiokeratoma, Incomplete RBBB
3 RF-03	M/47	*P205T*	Angiokeratoma, acroparesthesia, diarrhea, bradycardia, LVH

LVH: left ventricular hypertrophy, RBBB: right bundle branch block.

Table 2: Results of uGL-3, kidney histology, kidney GL-3 (LC/MS) and e-GFR by subject #

Subject	Sex/ age	Amino acid change	uGL-3 (foldxULN)	Kidney GL-3 histology				Kidney GL-3 content	e-GFR
				Podocytes	Distal tubules	Interstitial capillaries		µg/g	mL/min /1.73 m²
				score[2]	score[2]	score[2]	BLISS-LM[3]		
4 01-01	F/62	P259R	2	3	1.5	0	0.4	1930	90
4 01-02	F/39	P259R	13	3	0	0	0.2	3380	85
4 02-01	F/59	P205T	10	2.5	2.5	0.5	0.3	5370	76
4 02-03	F/36	*C52G*	normal	3	0	0	0.2	364	108
4 03-01	F/42	*R227X*	8	3	0	0	0.1	707	73
4 03-02	F/36	R112H	normal	1.5	0	0	0.2	293	101
4 03-05	F/43	L32P	8	3	0.5	0	0.2	1400	116
4 04-02	F/44	*E358K*	5	2	0	0	0.2	732	80
4 06-03	F/47	*M1I*	3	3	3	0	0.1	1630	83
2 01-02	M/27	*L415P*	28	3	1	1	2.3	4170	144
2 01-03	M/23	P259R	44	3	1.5	1	5.9	9510	127
2 01-04	M/18	P259R	31	3	2.5	1	2.9	9770	156
2 02-02	M/65	R301Q	4	3	3	0	0.3	4140	33
3 03-01	M/39	F295C	52	3	0	0	1.4	3500	119
3 03-02	M/31	C94S	48	3	0.5	1	2.6	2100	150
3 03-03	M/36	R112C	20	3	2.5	1	2.9	621	105
3 RF-01	M/55	N215S	2	3	1	0	0.3	1340	92
3 RF-03	M/47	P205T	15	n/a	0.5	n/a	n/a	5300	170

Figure 1: BLISS IC GL-3 versus $\log_{10}$ of kidney homogenate total GL-3 content (µg/g)



R=0.52 (Pearson Correlation)
R=0.56 (Spearman Rank correlation)
Shaded area represents 95% CI of the fitted line

Summary

- The mean age of subjects was 41.5 (range 18 to 65).
- 17/18 patients had a missense mutation.
- Patient 2 02-02 had renal insufficiency (e-GFR 33 mL/mn/1.73 m2).
- 7/18 subjects had proteinuria.
- Urinary GL-3 was elevated in 16/18 (range 2 to 52 fold above ULN).
- On semi-quantitative histology [2], 14/17 patients had the highest podocyte score of '3'. Distal tubular cells, which are more difficult to evaluate as they are less consistently present on the biopsy slide, were the second most affected: 5/18 patients were either '2' or '3'. For PTCs, 11/17 biopsies had a score of '0' and 6/17 of '1'.
- Scores for GL-3 inclusions per PTC via BLISS-LM varied from 0.1 to 5.9.
- The correlation between BLISS-LM PTC inclusions and GL3 tissue content was poor (R=0.52), suggesting that most GL-3 that is measured in homogenized tissue represents GL-3 accumulation in podocytes and tubular cells.

Discussion

GL-3 content is an important biochemical marker of Fabry disease that is directly linked to the pathophysiology of the disease. Further it is considered relevant to the evaluation of treatment efficacy.

Data from our study indicates that there is poor correlation between the different methods used to measure GL-3 in kidney as they seem to give different results. There is also not a good concordance between the GL-3 content of different cells within the kidney. In our study, while podocytes were severely affected, the amount of vascular GL-3 in PTCs was limited. This discrepancy was seen both in males and females, despite the fact that their FD could be considered clinically symptomatic or 'typical'. One of the subjects described had a severe renal insufficiency and high GL-3 loads in podocytes and distal tubular cells but a minimal amount of GL-3 in PTCs. The minimal GL-3 load seen in subject enrolled in these three phase 2 studies is at odd with previous publications, where the vascular component of FD was hypothesized to play a central role in the manifestation of the disease, including its renal manifestations [2, 4]. This probably indicates that FD is more heterogeneous that previously thought.

Recent works have highlighted the central role played by the podocytes in the pathophysiology of FD [5]. A central role played by the podocyte in Fabry nephropathy would be similar to other glomerulopathies and is consistent with the fact that many FD subjects develop proteinuria and, subsequently, progressive renal failurc. We suggest that in FD, proteinuria associated with increase urine GL-3 may signal increased podocyte GL-3 load and dysfunction.

Future work should focus on how best to evaluate podocytes function in patients with FD and how to better evaluate the activity of treatment to clear GL-3 from podocytes.

References

1. Fuller M, Sharp PC, Rozaklis T, et al. Urinary lipid profiling for the identification of Fabry hemizygotes and heterozygotes. Clin Chem 2005;51:688-94.
2. Thurberg BL, Rennke H, Colvin RB, et al. Globotriaosylceramide accumulation in the Fabry kidney is cleared from multiple cell types after enzyme replacement therapy. Kidney Int 2002: 62:1933-1946.
3. Barisoni LMC, Jennette JC, Colvin RB, et al. Novel Quantitative Virtual Microscopy-Based method to evaluate GL-3 inclusions in renal peritubular capillaries in patients with Fabry disease. Arch Pathol Lab Med (in press).
4. Desnick RJ, Ionnou YA, Eng CM: α -Galactosidase A deficiency: Fabry disease, in The Metabolic and Molecular Bases of Inherited Disease (8th ed, vol 3), edited by Scriver CR, Beaudet AL, Sly WS, Valle D, New York, McGraw-Hill, pp 3733–3774, 2001
5. Najafian B, Svarstad E, Bostad L, et al. Progressive podocyte injury and globotriaosylceramide (GL-3) accumulation in young patients with Fabry disease. Kidney Int 2011;79:663-70.