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Supplementary Data:

Global Glycosphingolipid Analysis in Urine and Plasma of Female Fabry Disease Patients

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Table S1. Table of mass spectral analysis parameters used in all 4 assays.

	Plasma Lyos-Gb3	Urine Lyso-Gb3	Plasma Gb3	Urine Gb3
column	Acquity UPLC BEH C18; Waters Corp.	Acquity UPLC BEH C8; Waters Corp.	Acquity UPLC BEH C8; Waters Corp.	Acquity UPLC BEH C8; Waters Corp.
Length	50 mm	50 mm	50 mm	50 mm
internal diameter	2.1 mm	2.1 mm	2.1 mm	2.1 mm
particle diameter	1.7 µM	1.7 µM	1.7 µM	1.7 µM
column temperature	40 °C	40 °C	40 °C	40 °C
Guard column		ACQUITY UPLC BEH C8 VanGuard Pre-column (130 Å, 1.7 µm, 2.1 mm X 5 mm)		
weak wash solvent	0.1% TFA	5% MeOH	5% MeOH	5% MeOH
strong wash solvent	IPA:ddH2O:MeOH:ACN (1:1:1:1)	100% MeOH	IPA:ddH2O:MeOH:ACN (1:1:1:1)	IPA:ddH2O:MeOH:ACN (1:1:1:1)
mobile phase A	0.1% FA	0.1% FA	0.1% FA	0.1% FA
mobile phase B	100% MeOH	0.1% FA in MeOH	100% MeOH	100% MeOH
Gradient	Gradient parameters 1	Gradient parameters 2	Gradient parameters 3	Gradient parameters 3
flow rate	0.6 mL/min	0.5 mL/min	0.6 mL/min	0.6 mL/min
injection volume	5 µL	10 µL	5 µL	5 µL
injection mode	partial loop	partial loop	partial loop	partial loop
autosampler temperature	10 °C	10 °C	10 °C	10 °C

Table S2: Gradient parameters 1

Time	Solvent A_1 (%)	Solvent B_2 (%)	Flow (ml/min)	Curve
0.00	80	20	0.600	Initial
0.20	80	20	0.600	6
5.00	0.1	99.9	0.600	6
8.00	0.1	99.9	0.600	6
8.01	80	20	0.600	1
10.00	80	20	0.600	1

Table S3: Gradient parameters 2

Time	Solvent A (%)	Solvent B (%)	Flow (ml/min)	Curve
0.00	95	5	0.500	Initial
0.50	95	5	0.500	6
1.00	0	100	0.500	6
2.50	0	100	0.600	6
2.51	95	5	0.600	1
3.50	95	5	0.500	1

Table S4: Gradient parameters 3

Time	Solvent A (%)	Solvent B (%)	Flow (ml/min)	Curve
0.00	50	50	0.600	Initial
0.50	50	50	0.600	6
1.5	1.0	99	0.600	6
2.5	1.0	99	0.600	6
2.51	50	50	0.600	1
3.50	50	50	0.600	1

Table S5: Mass transitions and conditions for all internal standards, GSLs, lyso-gb3 and analogues used in this study

Analyte	Precursor m/z	Product m/z	Cone voltage	Collision energy
CMH_C16:3	716.4	554.5	125	42
CMH_C16:2	718.4	556.5	125	42
CMH_C16:1	720.5	558.5	125	42
CMH_C16:0	722.5	560.5	125	42
CMH_C16:0-D3 IS	725.7	563.7	78	42
CMH_C16:3 -OH	732.4	570.5	125	42
CMH_C16:2-OH	734.4	572.5	125	42
CMH_C16:1-OH	736.5	574.5	125	42
CMH_C16:0-OH	738.5	576.5	125	42
CMH_C18:3	744.5	582.5	125	42
CMH_C18:2	746.5	584.5	125	42
CMH_C18:1	748.5	586.5	125	42
CMH_C18:0	750.5	588.6	125	42
CMH_C18:3 -OH	760.5	598.5	125	42
CMH_C18:2-OH	762.5	600.5	125	42
CMH_C18:1-OH	764.5	602.5	125	42
CMH_C18:0-OH	766.5	604.6	125	42
CMH_C20:3	772.5	610.6	125	42
CMH_C20:2	774.6	612.6	125	42
CMH_C20:1	776.6	614.6	125	42
CMH_C20:0	778.6	616.6	125	42
CMH_C20:3 -OH	788.5	626.6	125	42
CMH_C20:2-OH	790.5	628.6	125	42
CMH_C20:1-OH	792.6	630.6	125	42
CMH_C20:0-OH	794.6	632.6	125	42
CMH_C22:3	800.6	638.6	125	42
CMH_C22:2	802.6	640.6	125	42
CMH_C22:1	804.6	642.7	125	42
CMH_C22:0	806.6	644.7	125	42
CMH_C22:3 -OH	816.6	654.6	125	42
CMH_C22:2-OH	818.6	656.6	125	42
CMH_C22:1-OH	820.6	658.6	125	42
CMH_C22:0-OH	822.6	660.7	125	42
CMH_C24:3	828.6	666.7	125	42
CMH_C24:2	830.7	668.7	125	42
CMH_C24:1	832.7	670.7	125	42
CMH_C24:0	834.7	672.7	125	42
CMH_C24:3 -OH	844.6	682.7	125	42
CMH_C24:2-OH	846.7	684.7	125	42
CMH_C24:1-OH	848.7	686.7	125	42

CMH_C24:0-OH	850.7	688.7	125	42
CMH_C26:3	856.7	694.7	125	42
CMH_C26:2	858.7	696.7	125	42
CMH_C26:1	860.7	698.8	125	42
CMH_C26:0	862.7	700.8	125	42
CMH_C26:3 -OH	872.7	710.7	125	42
CMH_C26:2-OH	874.7	712.7	125	42
CMH_C26:1-OH	876.7	714.8	125	42
CMH_C26:0-OH	878.7	716.8	125	42
	0	0		
CDH_C16:3	878.2	716.2	124	57
CDH_C16:2	880.2	718.2	124	57
CDH_C16:1	882.2	720.3	124	57
CDH_C16:0	884.2	722.3	124	57
CDH_C16-d3	887.3	725.3	124	57
CDH_C16:3 -OH	894.2	732.2	124	57
CDH_C16:2-OH	896.2	734.2	124	57
CDH_C16:1-OH	898.2	736.2	124	57
CDH_C16:0-OH	900.2	738.3	124	57
CDH_C18:3	906.2	744.3	124	57
CDH_C18:2	908.3	746.3	124	57
CDH_C18:1	910.3	748.3	124	57
CDH_C18:0	912.3	750.3	124	57
CDH_C18:3 -OH	922.2	760.3	124	57
CDH_C18:2-OH	924.3	762.3	124	57
CDH_C18:1-OH	926.3	764.3	124	57
CDH_C18:0-OH	928.3	766.3	124	57
CDH_C20:3	934.3	772.3	124	57
CDH_C20:2	936.3	774.3	124	57
CDH_C20:1	938.3	776.4	124	57
CDH_C20:0	940.3	778.4	124	57
CDH_C20:3 -OH	950.3	788.3	124	57
CDH_C20:2-OH	952.3	790.3	124	57
CDH_C20:1-OH	954.3	792.4	124	57
CDH_C20:0-OH	956.3	794.4	124	57
CDH_C22:3	962.3	800.4	124	57
CDH_C22:2	964.4	802.4	124	57
CDH_C22:1	966.4	804.4	124	57
CDH_C22:0	968.4	806.4	124	57
CDH_C22:3 -OH	978.3	816.4	124	57
CDH_C22:2-OH	980.4	818.4	124	57
CDH_C22:1-OH	982.4	820.4	124	57
CDH_C22:0-OH	984.4	822.4	124	57
CDH_C24:3	990.4	828.4	124	57

CDH_C24:2	992.4	830.4	124	57
CDH_C24:1	994.4	832.5	124	57
CDH_C24:0	996.5	834.5	124	57
CDH_C24:3 -OH	1006.4	844.4	124	57
CDH_C24:2-OH	1008.4	846.4	124	57
CDH_C24:1-OH	1010.4	848.5	124	57
CDH_C24:0-OH	1012.4	850.5	124	57
CDH_C26:3	1018.5	856.5	124	57
CDH_C26:2	1020.5	858.5	124	57
CDH_C26:1	1022.5	860.5	124	57
CDH_C26:0	1024.5	862.5	124	57
CDH_C26:3 -OH	1034.4	872.5	124	57
CDH_C26:2-OH	1036.5	874.5	124	57
CDH_C26:1-OH	1038.5	876.5	124	57
CDH_C26:0-OH	1040.5	878.5	124	57
	0	0		
Gb3_C16:3	1040.6	878.6	124	64
Gb3_C16:2	1042.6	880.6	124	64
Gb3_C16:1	1044.6	882.6	124	64
Gb3_C16:0	1046.6	884.7	124	64
Gb3_C16:3 -OH	1056.6	894.6	124	64
Gb3_C16:2-OH	1058.6	896.6	124	64
Gb3_C16:1-OH	1060.6	898.6	124	64
Gb3_C16:0-OH	1062.6	900.7	124	64
Gb3_C17	1060.7	898.8	136	64
Gb3_C18:3	1068.6	906.7	124	64
Gb3_C18:2	1070.7	908.7	124	64
Gb3_C18:1	1072.7	910.7	124	64
Gb3_C18:0	1074.7	912.7	124	64
C18:0-D3-GB3	1077.8	915.9	108	64
Gb3_C18:3 -OH	1084.6	922.7	124	64
Gb3_C18:2-OH	1086.6	924.7	124	64
Gb3_C18:1-OH	1088.7	926.7	124	64
Gb3_C18:0-OH	1090.7	928.7	124	64
Gb3_C20:3	1096.7	934.7	124	64
Gb3_C20:2	1098.7	936.7	124	64
Gb3_C20:1	1100.7	938.8	124	64
Gb3_C20:0	1102.7	940.8	124	64
Gb3_C20:3 -OH	1112.7	950.7	124	64
Gb3_C20:2-OH	1114.7	952.7	124	64
Gb3_C20:1-OH	1116.7	954.7	124	64
Gb3_C20:0-OH	1118.7	956.8	124	64
Gb3_C22:3	1124.7	962.8	124	64
Gb3_C22:2	1126.8	964.8	124	64

Gb3_C22:1	1128.8	966.8	124	64
Gb3_C22:0	1130.8	968.8	124	64
Gb3_C22:3 -OH	1140.7	978.8	124	64
Gb3_C22:2-OH	1142.8	980.8	124	64
Gb3_C22:1-OH	1144.8	982.8	124	64
Gb3_C22:0-OH	1146.8	984.8	124	64
Gb3_C24:3	1152.8	990.8	124	64
Gb3_C24:2	1154.8	992.8	124	64
Gb3_C24:1	1156.8	994.9	124	64
Gb3_C24:0	1158.8	996.9	124	64
Gb3_C24:3 -OH	1168.8	1006.8	124	64
Gb3_C24:2-OH	1170.8	1008.8	124	64
Gb3_C24:1-OH	1172.8	1010.9	124	64
Gb3_C24:0-OH	1174.8	1012.9	124	64
Gb3_C26:3	1180.8	1018.9	124	64
Gb3_C26:2	1182.9	1020.9	124	64
Gb3_C26:1	1184.9	1022.9	124	64
Gb3_C26:0	1186.9	1024.9	124	64
Gb3_C26:3 -OH	1196.8	1034.9	124	64
Gb3_C26:2-OH	1198.9	1036.9	124	64
Gb3_C26:1-OH	1200.9	1038.9	124	64
Gb3_C26:0-OH	1202.9	1040.9	124	64
	0	0		
Gb4 C16:3	1243.4	1040.4	125	52
Gb4 C16:2	1245.4	1042.4	125	52
Gb4 C16:1	1247.4	1044.4	125	52
Gb4 C16:0	1249.4	1046.5	125	52
Gb4 C16:3 -OH	1259.4	1056.4	125	52
Gb4 C16:2-OH	1261.4	1058.4	125	52
Gb4 C16:1-OH	1263.4	1060.4	125	52
Gb4 C16:0-OH	1265.4	1062.5	125	52
Gb4 C18:3	1271.4	1068.5	125	52
Gb4 C18:2	1273.5	1070.5	125	52
Gb4 C18:1	1275.5	1072.5	125	52
Gb4 C18:0	1277.5	1074.5	125	52
Gb4 C18:3 -OH	1287.4	1084.5	125	52
Gb4 C18:2-OH	1289.5	1086.5	125	52
Gb4 C18:1-OH	1291.5	1088.5	125	52
Gb4 C18:0-OH	1293.5	1090.5	125	52
Gb4 C20:3	1299.5	1096.5	125	52
Gb4 C20:2	1301.5	1098.5	125	52
Gb4 C20:1	1303.5	1100.5	125	52
Gb4 C20:0	1305.5	1102.6	125	52
Gb4 C20:3 -OH	1315.5	1112.5	125	52

Gb4 C20:2-OH	1317.5	1114.5	125	52
Gb4 C20:1-OH	1319.5	1116.5	125	52
Gb4 C20:0-OH	1321.5	1118.6	125	52
Gb4 C22:3	1327.5	1124.6	125	52
Gb4 C22:2	1329.6	1126.6	125	52
Gb4 C22:1	1331.6	1128.6	125	52
Gb4 C22:0	1333.6	1130.6	125	52
Gb4 C22:3 -OH	1343.5	1140.6	125	52
Gb4 C22:2-OH	1345.6	1142.6	125	52
Gb4 C22:1-OH	1347.6	1144.6	125	52
Gb4 C22:0-OH	1349.6	1146.6	125	52
Gb4 C24:3	1355.6	1152.6	125	52
Gb4 C24:2	1357.6	1154.6	125	52
Gb4 C24:1	1359.6	1156.7	125	52
Gb4 C24:0	1361.7	1158.7	125	52
Gb4 C24:3 -OH	1371.6	1168.6	125	52
Gb4 C24:2-OH	1373.6	1170.6	125	52
Gb4 C24:1-OH	1375.6	1172.6	125	52
Gb4 C24:0-OH	1377.6	1174.7	125	52
Gb4 C26:3	1383.7	1180.7	125	52
Gb4 C26:2	1385.7	1182.7	125	52
Gb4 C26:1	1387.7	1184.7	125	52
Gb4 C26:0	1389.7	1186.7	125	52
Gb4 C26:3 -OH	1399.7	1196.7	125	52
Gb4 C26:2-OH	1401.7	1198.7	125	52
Gb4 C26:1-OH	1403.7	1200.7	125	52
Gb4 C26:0-OH	1405.7	1202.7	125	52
IS- dimethylpsychosine	490.2	292.31	120	24
Lyso-Gb3	786.4575	282.303	4	30
Lyso-Gb3 (+50)	836.45	350.24	50	35
Lyso-Gb3 (+34)	820.45	334.25	60	32
Lyso-Gb3 (+18)	804.46	318.25	65	30
Lyso-Gb3 (+16)	802.44	280.26	65	38
Lyso-Gb3 (-2)	784.43	280.26	65	35
Lyso-Gb3 (-28)	758.42	254.25	55	35

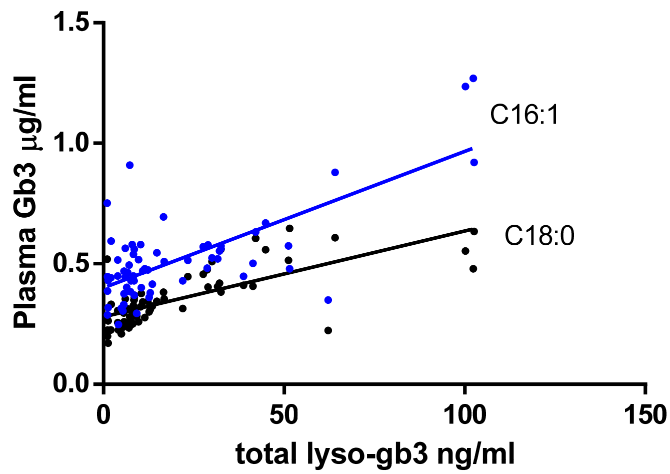
Additional information on determination of GSL concentrations and QC data:

Peak integration was performed using Waters Targetlynx software. By visual evaluation, a value of 0 was assigned if there was no visible peak or the value was below a signal to noise value of 3:1. Values were normalised to appropriate internal standard assuming identical response of isoforms (C17-CTH for Gb3 and Gb4, *d3*-C16-Lactosylceramide for CDH and CMH, dimethylpsychosine for lyso-Gb3). CMH, CDH, Gb3 and GB4 concentrations were calculated based on known amount of the IS used as a response factor. Lyso-Gb3 concentrations were calculated using a standard linear calibration line.

Intra-batch precision was determined by repeat injection through the run of three QCs of a non-Fabry, low gb3 level Fabry patient sample and high gb3 level Fabry patient sample.

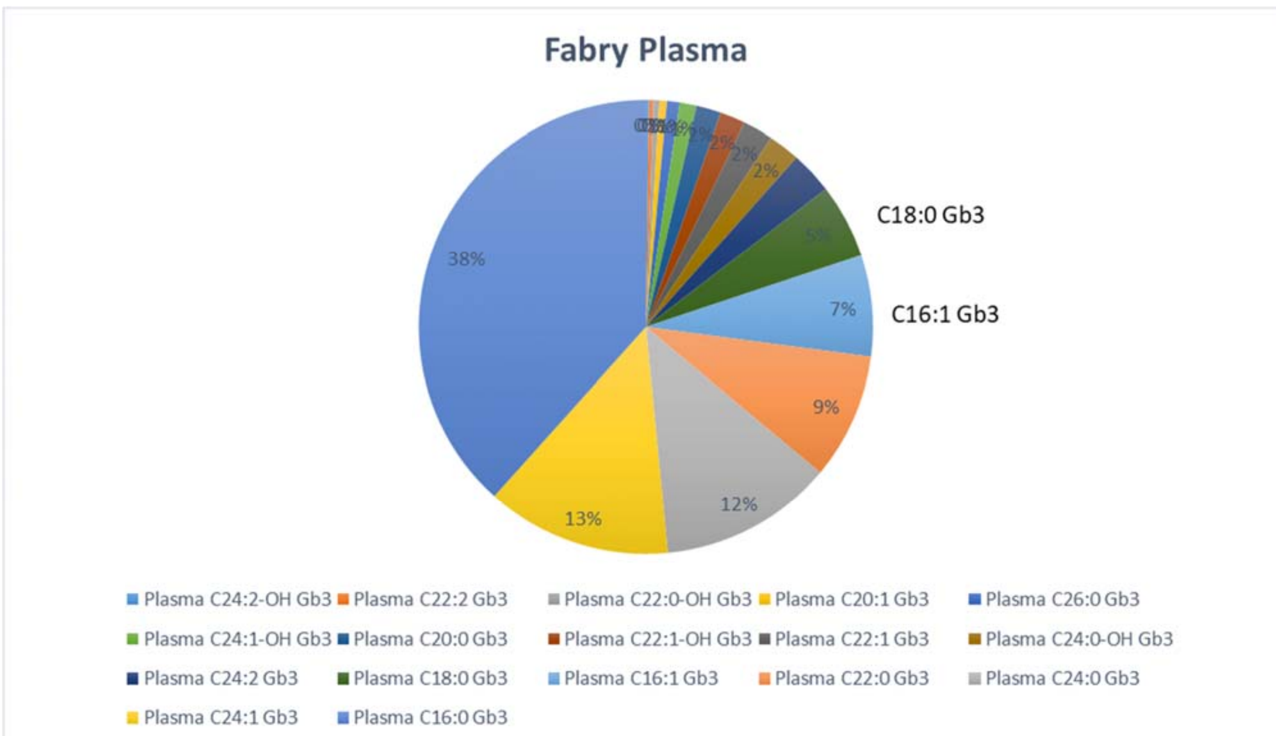
Table S6: Coefficient of variation values for 3 QC samples run throughout the assay.

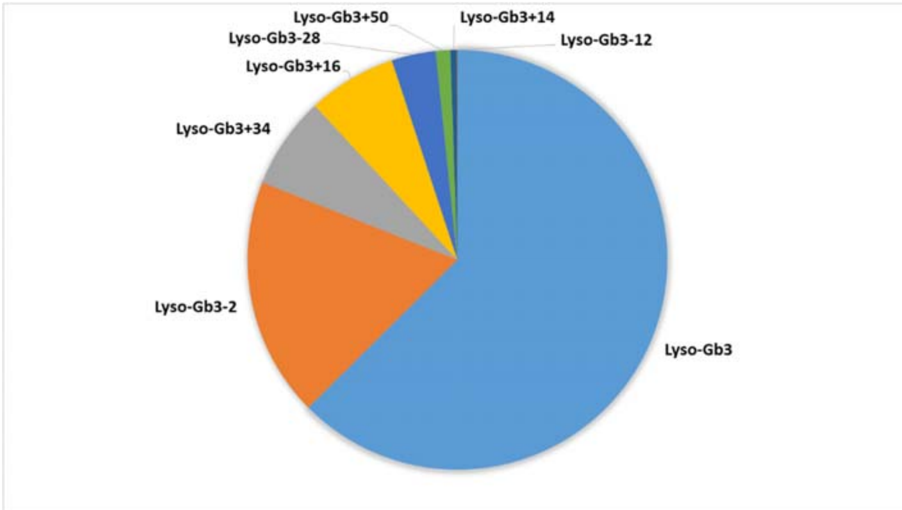
Plasma/Urine GSL	**CQC CV%**	**LQC CV%**	**HQC CV%**
Plasma Gb2	4.93	1.35	3.31
Plasma Gb3	4.08	3.64	6.06
Urine Gb2	5.43	5.00	3.38
Urine Gb3	8.28	3.37	1.17
Plasma Lyso-Gb3	6.72	3.90	3.30
Urine Lyso-Gb3	Below LOD	Below LOD	3.56



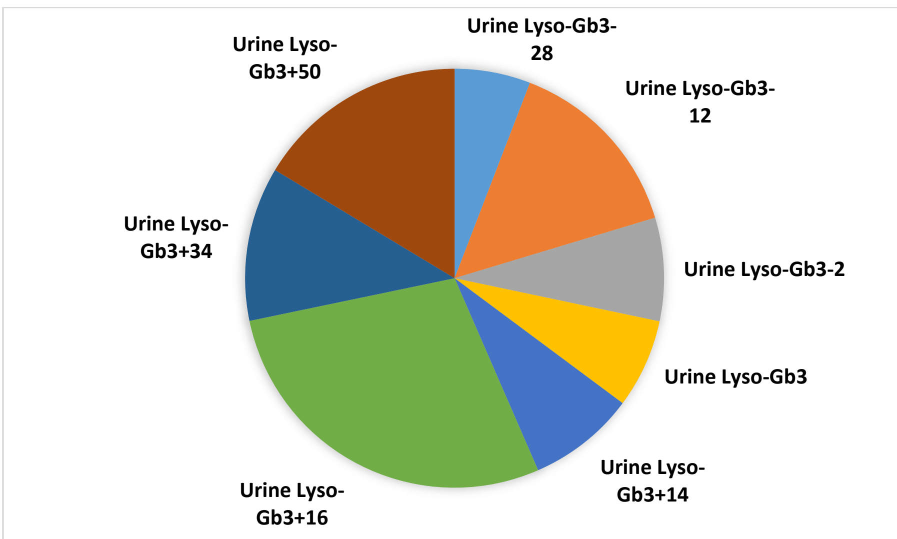
Supplementary figure S1. Individual Pearson correlation analysis of the Gb3 isoforms C16:1 and C18:0 that have the strongest (r2>0.7, p<0.0001) relationship with total plasma lyso-Gb3.

A. Plasma





B. Plasma



C. Urine

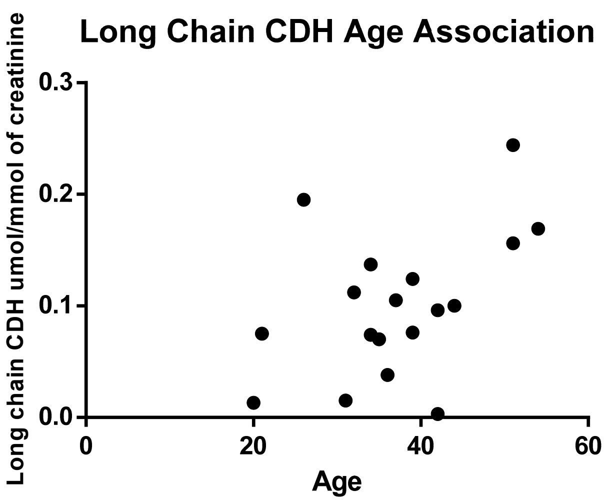
Supplementary figure S2. A Top panel shows plasma gb3 isoform composition of mean values of isoforms from all Fabry patients. Isoforms that have strong association with lyso-gb3 are indicated in the pie chart and are not the most abundant gb3 isoforms. **B** shows the plasma and **C** urine lyso-Gb_3 analogues composition of all Fabry patient samples in this study.

Table S6. Multivariate statistical analysis. OPLS-DA parameters for Fabry female vs Female controls.

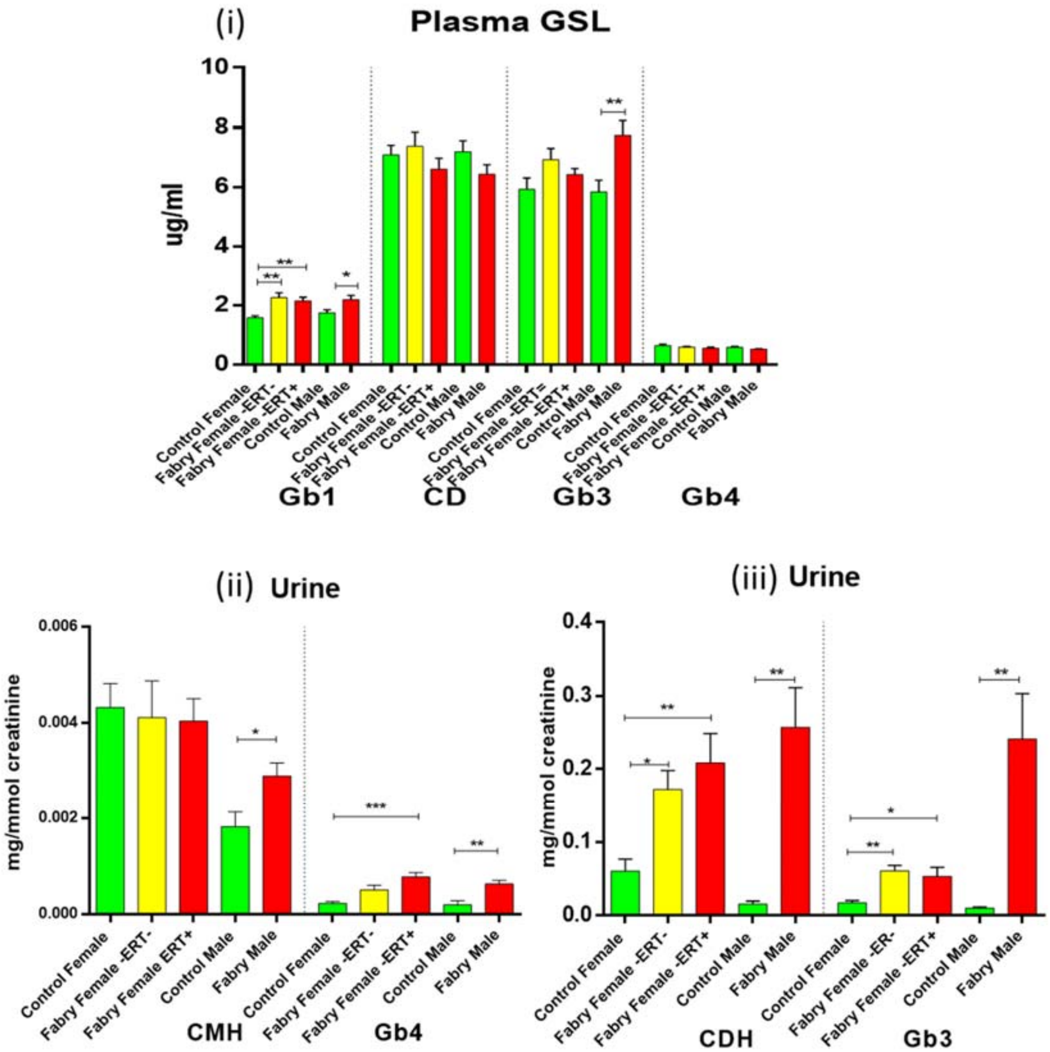
Name	type	A	N	R^2X	R^2Y	Q^2Y	p
Control Female vs Fabry Female Urine	OPLS-DA	1+1+0	49	0.45	0.64	0.39	0.00
Control Female vs Fabry Female Plasma	OPLS-DA	1+3+0	65	0.75	0.98	0.97	>0.0

A = number of components, N = number of samples that the model is based on, R^2 "goodness of fit" parameter that shows how well the model describes the variation in the data. R^2X, R^2Y are the cumulative variations explained in the metabolite and class-variable data respectively, Q^2Y "goodness of prediction" parameter and is the cross-validated prediction estimate of class separation that shows how well samples are predicted by the model

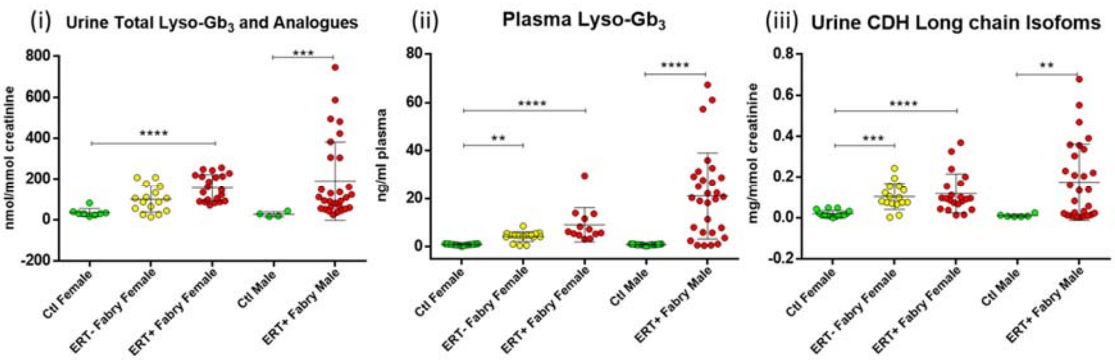
p values were obtained using CV-ANOVA in SIMCA 15.0



Supplementary figure S3. Age association of long chain CDG isoforms in the asymptomatic Fabry female group. No significant correlation with age is observed. Spearman correlation analysis n= 17, r^2= 0.39 p<0.1



Supplementary figure S4. **Comparison of each GSL species in Fabry patients according to ERT status for female samples**. (i) Mean + SEM plasma values of CMH, CDH, Gb_3 and Gb_4. Significance in the Fabry female group for plasma is determined by one way ANOVA and for Fabry males by unpaired t-test (ii) Mean + SEM urine values for the lower abundant GSL species CMH and Gb_4 in urine. (iii) Mean + SEM urine values for the higher abundant GSL species CDH and Gb_3 in urine. Significance of urine GSLs was determined by use of non-parametric Kruskal Wallis for the Fabry female groups and Mann-Witney test for the Fabry male group comparison. * $p<0.05$, ** $p<0.01$, *** $p<0.001$. Grouping the female patients by ERT (figure S4) as opposed to symptomatic status does not affect the plasma results observed in figure 3. For the urine GSLs CMH is unaffected, Gb4 ERT- is no longer significant whilst ERT+ increases in significance. CDH does not change by grouping by ERT instead of symptomatic status both female as both FD groups are still significant. For Gb3 the female ERT- group is more significant whilst the ERT+ is less significant than if grouped by symptomatic status.



Supplementary figure S5. Comparative analysis of Fabry patients grouped accrding ot ERT status for (i) total urine lyso-Gb3 plus analogues, (ii) plasma lyso-Gb3 and (iii) urine CDH long chain isoforms.