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Poster 303

Diagnostic strategy for Females suspected of Fabry Disease

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Introduction

Fabry disease (FD) is a progressive, X-linked inherited lysosomal storage disorder caused by genetic variants in the α-Galactosidase A gene (*GLA*). Partial or complete deficiency of the enzyme α-Galactosidase A (α-Gal A) results in a progressive accumulation of lipids with terminal α-Galactosyl residues, primarily globotriaosylceramide (Gb3, GL-3) and its deacylated derivative Lyso-GL-3 (lyso-Gb3) and leads to organ damage. Early diagnosis is vital to prevent clinical complications.

Due to random X-inactivation, α-Gal A activity in heterozygous females is not conclusive as women often present with normal enzyme levels, therefore potentially affected individuals might be missed. In contrast, primarily genetic testing leads to the identification of variants of unknown significance (VUS), and to frequent identification of benign variants such as p.D313Y and p.A143T.

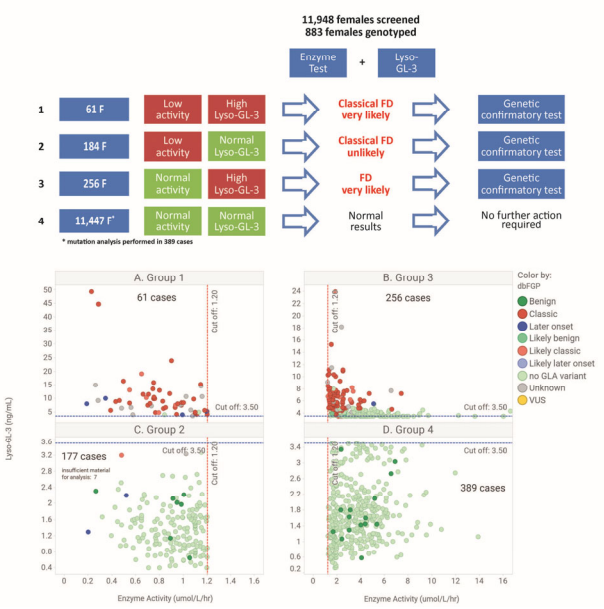
In recent years, Lyso-GL-3 has emerged as a useful biomarker in FD even allowing to differentiate between classic and later-onset FD. However, Lyso-GL-3 is not solely specific to FD, so diagnosis cannot be based on Lyso-GL-3 elevations alone. A subsequent genetic assessment is mandatory.

Study Cohort

A cohort of female patients suspected of Fabry disease where tested by a combined biochemical and genetic testing approach. 11,948 females were screened for α-Gal A enzyme activity and Lyso-GL-3 concentration from Dried Blood Spots (DBS).

Subjects were split into 4 groups based on enzyme activity and Lyso-GL-3 levels. Low α-Gal A activity is defined as equal to or below 1.2 µmol/L/hr. High Lyso-GL-3 is defined as equal to or above 3.5 ng/mL. Normal α-Gal A activity is above 1.2 µmol/L/hr and normal Lyso-GL-3 is below 3.5 ng/mL. In total, 883 females were genotyped, 389 that tested negative for both biochemical parameters and 494 that tested positive for at least one parameter.

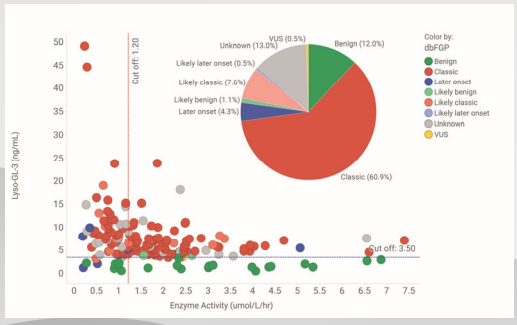
Summary of biochemical and genetic analysis



Group	# of cases		Enzyme Activity	Lyso-GL-3	A. Median enzyme activity (µmol/L/h) B. Median Lyso-GL-3 (ng/mL)			
							#.	A.	B.
1	61	97% PPV†	Low	High	Classic	missense	23	0.77	8.80
						nonsense	9	0.85	7.51
						inframe indel	2	0.34	29.27
						splice site	2	0.94	16.19
					Likely classic	missense	6	0.74	8.90
					Later onset	missense	4	0.67	6.68
					Unknown	missense	6	0.86	7.60
						nonsense	7	0.97	7.59
					no mutation		2	0.99	4.46
2	177	6% PPV†	Low	Normal			#.	A.	B.
					Likely classic	missense	1	0.48	3.20
					Later onset	missense	2	0.36	1.75
					Benign	missense	7*	0.95	2.02
					Unknown	missense	1	1.02	3.24
					no mutation		166	0.96	1.33
					insufficient material for analysis: 7				
3	256	39% PPV†	Normal	High			#.	A.	B.
					Classic	missense	50	1.82	5.74
						nonsense	22	2.01	6.18
						inframe indel	1	1.38	6.65
						splice site	3	2.03	6.15
					Likely classic	missense	7	2.37	6.71
					Later onset	missense	2	3.71	5.13
					Likely later onset	missense	1	2.38	3.85
					VUS	missense	1	2.43	3.60
					Likely benign	missense	2*	2.09	3.89
					Unknown	missense	7	2.37	5.89
						nonsense	1	2.95	5.05
						inframe indel	2	1.60	6.85
					no mutation		157	4.12	3.70
4	389	100% NPV‡	Normal	Normal	Benign	missense	15*	3.98	1.60
					no mutation		374	3.47	1.62
Total: 883									

†Positive predictive value
‡Negative predictive value
* Cases submitted per physician request (23 in total, 22 cases of Benning and 1 case of Likely benign phenotype)

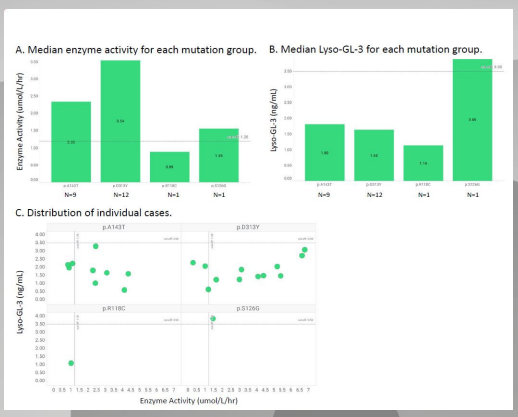
Distribution of genotyped samples



- 184 out of 883 females have been identified with one or two *GLA* variants
- 84 distinct sequence variants were classified by phenotype[1]
- 19 unique genetic variants (24 patients in total) was not found in International FD database[1]
- 23 samples were genotyped on physician request (all Benign variants)

- Genetic analysis was done on the same DBS sample as the enzyme activity and Lyso-GL-3 analysis

Distribution of 23 females genotyped per request



Genetic analysis revealed only benign variants p.D313Y, p.A143T, p.R118C, and one likely benign variant p.S126G.

- 9 cases with p.A143T
  - 6 showed normal enzyme activity and Lyso-GL-3 values.
  - 3 had low enzyme activity but still normal Lyso-GL-3 levels
- 12 cases with p.D313Y
  - 9 showed normal enzyme activity and Lyso-GL-3 values
  - 3 had low enzyme and normal Lyso-GL-3 levels
- One sample with p.R118C showed low enzyme activity and a normal Lyso-GL-3 value.
- One sample with p.S126G
  - showed a slight elevation of Lyso-GL-3 (3.89 ng/ml)
  - borderline enzyme activity (1.55 µmol/L/hr)

Conclusion

Testing females suspicious of FD using enzyme activity, together with the concentration of Lyso-GL-3 (lyso-Gb3) biomarker, substantially improved the diagnostic detection of Fabry disease in females compared to the enzyme activity alone. Abnormal values for both were highly suspicious of Fabry disease (97% PPV, similar to PPV in males). In cases with one abnormal biochemical value, elevated Lyso-GL-3 is a far more important indicator than low enzyme activity (39% PPV versus 6% PPV). Cases with clearly negative results for both biochemical parameters are unlikely to have Fabry disease, even in clinically highly suspicious cases.

Reference:

[1] Balendran S, Oliva P, Sansen S, Mechtler TP, Streubel B, Cobos PN, Lukacs Z, Kasper DC. Diagnostic strategy for females suspected of Fabry disease. Clin Genet. 2019 Dec 20. doi: 10.1111/cge.13694. [Epub ahead of print] PubMed PMID:31860127.

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