

Methods to detect cross-reactivity of anti- α -galactosidase A IgG antibody produced against other enzyme replacement therapy to pegunigalsidase in FD patient's serum

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Introduction:

Enzyme Replacement Therapy (ERT) is the gold standard treatment for Fabry disease (FD) and is indicated for all patients. Several ERT options are available worldwide and have demonstrated efficacy in slowing disease progression, reducing Lyso-GL3, stabilizing organ function, and alleviating symptoms. However, as biologic therapies, these medicaments can induce the formation of antidrug antibodies (ADAs), mainly IgG, which may affect therapeutic efficacy. The aim of this study was to develop and compare different ELISA methods to quantify ADAs in serum samples from FD patients treated with ERT and to assess potential cross-reactivity between the available enzymes.

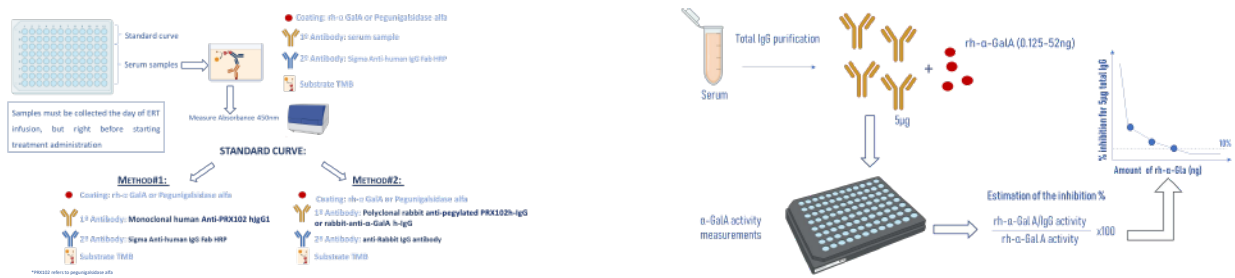
Methods:

Detection of anti-ERT-IgG:

We measured total IgG against α -Galactosidase A (α -GalA) and compared the results extrapolated from standard curves prepared with different reference antibodies. In Method #1 we used a human anti pegunigalsidase alfa-IgG antibody as reference for all the enzymes, while in Method #2 we used a rabbit-anti-pegylated- α -GalA-IgG antibody to capture pegunigalsidase alfa and a rabbit-anti-human- α -GalA-IgG antibody to bind recombinant-human- α -Gal A produced in CHO cells (rh- α -GalA).

Detection of Neutralizing anti-ERT-IgG:

A fixed amount of 5 μ g of total IgG, purified from patient serum, were incubated with increasing concentrations of either pegunigalsidase alfa or rh- α -Gal A and enzymatic activity was measured using 4-Methyl-umbelliferil- α -galactopyranoside as substrate (μ M)



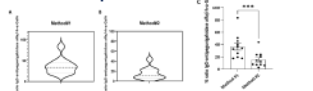
Samples were obtained by Vigo Hospital Biobank and 2 additional national centers (nº B.0000802, Spanish National registry of Biobanks and CEIm-G nº 2023-542). Patients features are summarized in the table.

Results:

Both methods proposed for anti-ERT-IgG assessment were reliable to detect ADAs in FD patients' serum, in response to either pegunigalsidase alfa or rh- α -GalA with a high sensitivity and specificity.

Sample	Age	Sex	ERT	Enzyme	Enzyme dose (mg/kg)	Enzyme frequency	Enzyme start date	Enzyme end date	Enzyme duration (years)
F1	41	M	ERT	Pegunigalsidase alfa	0.4	Bi-weekly	2015	2023	8
F2	41	M	ERT	Pegunigalsidase alfa	0.4	Bi-weekly	2015	2023	8
F3	41	M	ERT	Pegunigalsidase alfa	0.4	Bi-weekly	2015	2023	8
F4	41	M	ERT	Pegunigalsidase alfa	0.4	Bi-weekly	2015	2023	8
F5	41	M	ERT	Pegunigalsidase alfa	0.4	Bi-weekly	2015	2023	8
F6	41	M	ERT	Pegunigalsidase alfa	0.4	Bi-weekly	2015	2023	8
F7	41	M	ERT	Pegunigalsidase alfa	0.4	Bi-weekly	2015	2023	8
F8	41	M	ERT	Pegunigalsidase alfa	0.4	Bi-weekly	2015	2023	8
F9	41	M	ERT	Pegunigalsidase alfa	0.4	Bi-weekly	2015	2023	8
F10	41	M	ERT	Pegunigalsidase alfa	0.4	Bi-weekly	2015	2023	8
F11	41	M	ERT	Pegunigalsidase alfa	0.4	Bi-weekly	2015	2023	8
F12	41	M	ERT	Pegunigalsidase alfa	0.4	Bi-weekly	2015	2023	8
F13	41	M	ERT	Pegunigalsidase alfa	0.4	Bi-weekly	2015	2023	8
F14	41	M	ERT	Pegunigalsidase alfa	0.4	Bi-weekly	2015	2023	8
F15	41	M	ERT	Pegunigalsidase alfa	0.4	Bi-weekly	2015	2023	8
F16	41	M	ERT	Pegunigalsidase alfa	0.4	Bi-weekly	2015	2023	8
F17	41	M	ERT	Pegunigalsidase alfa	0.4	Bi-weekly	2015	2023	8
F18	41	M	ERT	Pegunigalsidase alfa	0.4	Bi-weekly	2015	2023	8
F19	41	M	ERT	Pegunigalsidase alfa	0.4	Bi-weekly	2015	2023	8
F20	41	M	ERT	Pegunigalsidase alfa	0.4	Bi-weekly	2015	2023	8
F21	41	M	ERT	Pegunigalsidase alfa	0.4	Bi-weekly	2015	2023	8
F22	41	M	ERT	Pegunigalsidase alfa	0.4	Bi-weekly	2015	2023	8
F23	41	M	ERT	Pegunigalsidase alfa	0.4	Bi-weekly	2015	2023	8
F24	41	M	ERT	Pegunigalsidase alfa	0.4	Bi-weekly	2015	2023	8
F25	41	M	ERT	Pegunigalsidase alfa	0.4	Bi-weekly	2015	2023	8
F26	41	M	ERT	Pegunigalsidase alfa	0.4	Bi-weekly	2015	2023	8
F27	41	M	ERT	Pegunigalsidase alfa	0.4	Bi-weekly	2015	2023	8
F28	41	M	ERT	Pegunigalsidase alfa	0.4	Bi-weekly	2015	2023	8
F29	41	M	ERT	Pegunigalsidase alfa	0.4	Bi-weekly	2015	2023	8
F30	41	M	ERT	Pegunigalsidase alfa	0.4	Bi-weekly	2015	2023	8
F31	41	M	ERT	Pegunigalsidase alfa	0.4	Bi-weekly	2015	2023	8
F32	41	M	ERT	Pegunigalsidase alfa	0.4	Bi-weekly	2015	2023	8
F33	41	M	ERT	Pegunigalsidase alfa	0.4	Bi-weekly	2015	2023	8
F34	41	M	ERT	Pegunigalsidase alfa	0.4	Bi-weekly	2015	2023	8
F35	41	M	ERT	Pegunigalsidase alfa	0.4	Bi-weekly	2015	2023	8
F36	41	M	ERT	Pegunigalsidase alfa	0.4	Bi-weekly	2015	2023	8
F37	41	M	ERT	Pegunigalsidase alfa	0.4	Bi-weekly	2015	2023	8
F38	41	M	ERT	Pegunigalsidase alfa	0.4	Bi-weekly	2015	2023	8
F39	41	M	ERT	Pegunigalsidase alfa	0.4	Bi-weekly	2015	2023	8
F40	41	M	ERT	Pegunigalsidase alfa	0.4	Bi-weekly	2015	2023	8
F41	41	M	ERT	Pegunigalsidase alfa	0.4	Bi-weekly	2015	2023	8
F42	41	M	ERT	Pegunigalsidase alfa	0.4	Bi-weekly	2015	2023	8
F43	41	M	ERT	Pegunigalsidase alfa	0.4	Bi-weekly	2015	2023	8
F44	41	M	ERT	Pegunigalsidase alfa	0.4	Bi-weekly	2015	2023	8
F45	41	M	ERT	Pegunigalsidase alfa	0.4	Bi-weekly	2015	2023	8
F46	41	M	ERT	Pegunigalsidase alfa	0.4	Bi-weekly	2015	2023	8
F47	41	M	ERT	Pegunigalsidase alfa	0.4	Bi-weekly	2015	2023	8
F48	41	M	ERT	Pegunigalsidase alfa	0.4	Bi-weekly	2015	2023	8
F49	41	M	ERT	Pegunigalsidase alfa	0.4	Bi-weekly	2015	2023	8
F50	41	M	ERT	Pegunigalsidase alfa	0.4	Bi-weekly	2015	2023	8
F51	41	M	ERT	Pegunigalsidase alfa	0.4	Bi-weekly	2015	2023	8
F52	41	M	ERT	Pegunigalsidase alfa	0.4	Bi-weekly	2015	2023	8
F53	41	M	ERT	Pegunigalsidase alfa	0.4	Bi-weekly	2015	2023	8
F54	41	M	ERT	Pegunigalsidase alfa	0.4	Bi-weekly	2015	2023	8
F55	41	M	ERT	Pegunigalsidase alfa	0.4	Bi-weekly	2015	2023	8
F56	41	M	ERT	Pegunigalsidase alfa	0.4	Bi-weekly	2015	2023	8
F57	41	M	ERT	Pegunigalsidase alfa	0.4	Bi-weekly	2015	2023	8
F58	41	M	ERT	Pegunigalsidase alfa	0.4	Bi-weekly	2015	2023	8
F59	41	M	ERT	Pegunigalsidase alfa	0.4	Bi-weekly	2015	2023	8
F60	41	M	ERT	Pegunigalsidase alfa	0.4	Bi-weekly	2015	2023	8
F61	41	M	ERT	Pegunigalsidase alfa	0.4	Bi-weekly	2015	2023	8
F62	41	M	ERT	Pegunigalsidase alfa	0.4	Bi-weekly	2015	2023	8
F63	41	M	ERT	Pegunigalsidase alfa	0.4	Bi-weekly	2015	2023	8
F64	41	M	ERT	Pegunigalsidase alfa	0.4	Bi-weekly	2015	2023	8
F65	41	M	ERT	Pegunigalsidase alfa	0.4	Bi-weekly	2015	2023	8
F66	41	M	ERT	Pegunigalsidase alfa	0.4	Bi-weekly	2015	2023	8
F67	41	M	ERT	Pegunigalsidase alfa	0.4	Bi-weekly	2015	2023	8
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F69	41	M	ERT	Pegunigalsidase alfa	0.4	Bi-weekly	2015	2023	8
F70	41	M	ERT	Pegunigalsidase alfa	0.4	Bi-weekly	2015	2023	8
F71	41	M	ERT	Pegunigalsidase alfa	0.4	Bi-weekly	2015	2023	8
F72	41	M	ERT	Pegunigalsidase alfa	0.4	Bi-weekly	2015	2023	8
F73	41	M	ERT	Pegunigalsidase alfa	0.4	Bi-weekly	2015	2023	8
F74	41	M	ERT	Pegunigalsidase alfa	0.4	Bi-weekly	2015	2023	8
F75	41	M	ERT	Pegunigalsidase alfa	0.4	Bi-weekly	2015	2023	8
F76	41	M	ERT	Pegunigalsidase alfa	0.4	Bi-weekly	2015	2023	8
F77	41	M	ERT	Pegunigalsidase alfa	0.4	Bi-weekly	2015	2023	8
F78	41	M	ERT	Pegunigalsidase alfa	0.4	Bi-weekly	2015	2023	8
F79	41	M	ERT	Pegunigalsidase alfa	0.4	Bi-weekly	2015	2023	8
F80	41	M	ERT	Pegunigalsidase alfa	0.4	Bi-weekly	2015	2023	8
F81	41	M	ERT	Pegunigalsidase alfa	0.4	Bi-weekly	2015	2023	8
F82	41	M	ERT	Pegunigalsidase alfa	0.4	Bi-weekly	2015	2023	8
F83	41	M	ERT	Pegunigalsidase alfa	0.4	Bi-weekly	2015	2023	8
F84	41	M	ERT	Pegunigalsidase alfa	0.4	Bi-weekly	2015	2023	8
F85	41	M	ERT	Pegunigalsidase alfa	0.4	Bi-weekly	2015	2023	8
F86	41	M	ERT	Pegunigalsidase alfa	0.4	Bi-weekly	2015	2023	8
F87	41	M	ERT	Pegunigalsidase alfa	0.4	Bi-weekly	2015	2023	8
F88	41	M	ERT	Pegunigalsidase alfa	0.4	Bi-weekly	2015	2023	8
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F95	41	M	ERT	Pegunigalsidase alfa	0.4	Bi-weekly	2015	2023	8
F96	41	M	ERT	Pegunigalsidase alfa	0.4	Bi-weekly	2015	2023	8
F97	41	M	ERT	Pegunigalsidase alfa	0.4	Bi-weekly	2015	2023	8
F98	41	M	ERT	Pegunigalsidase alfa	0.4	Bi-weekly	2015	2023	8
F99	41	M	ERT	Pegunigalsidase alfa	0.4	Bi-weekly	2015	2023	8
F100	41	M	ERT	Pegunigalsidase alfa	0.4	Bi-weekly	2015	2023	8

Methods comparison



The two methods present a significant difference in the estimation of the affinity of ADAs against the evaluated drugs. Method#1, which use a monoclonal reference antibody, is more reliable to compare and do not overestimate the differences between pegunigalsidase alfa and rh-