ESC HEART FAILURE
ESC Heart Failure 2020; **7**: 1331–1337
Published online 29 April 2020 in Wiley Online Library (wileyonlinelibrary.com) **DOI:** 10.1002/ehf2.12723

CASE REPORT

Fabry cardiomyopathy: Gb3-induced auto-reactive panmyocarditis requiring heart transplantation

Andrea Frustaci[1]*, Maurizio Scarpa[2], Rosalia Maria da Riol[2], Chiara Agrati[3], Nicoletta Finato[4], Romina Verardo[5], Claudia Grande[5], Cristina Chimenti[1], Concetta Nora[6], Matteo Antonio Russo[7,8] and Ugolini Livi[6]

[1]*Department of Clinical, Internal, Anesthesiologist and Cardiovascular Sciences, La Sapienza University, Rome, Italy;* [2]*Regional Coordinating Center for Rare Diseases, Udine University Hospital, Rome, Italy;* [3]*Cellular Immunology Laboratory, INMI L Spallanzani, Rome, Italy;* [4]*Department of Medicine, Pathologic Anatomy Unit, University of Udine, Rome, Italy;* [5]*Cellular and Molecular Cardiology Lab, IRCCS L. Spallanzani, Rome, Italy;* [6]*Cardiothoracic Surgery, Udine University Hospital, Rome, Italy;* [7]*MEBIC Consortium, San Raffaele Open University, Rome, Italy;* [8]*Laboratory of Molecular and Cellular Pathology, IRCCS San Raffaele Pisana, Rome, Italy*

Abstract

Resistance to enzyme replacement therapy (ERT) is a major therapeutic challenge in Fabry disease (FD). Recent reports attribute to immune-mediated inflammation a main role in promoting disease progression and resistance to ERT. Aim of the study is to report a Gb3-induced auto-reactive panmyocarditis causing inefficacy of ERT and severe electrical instability, which required cardiac transplantation. Examining the explanted heart from a 57-year-old man with FD cardiomyopathy (CM) on 3-year ERT presenting incoming ventricular fibrillation, we documented a severe virus-negative myocarditis extended to cardiomyocytes, intramural coronary vessels, conduction tissue, and subepicardial ganglia. Serology was positive for anti-Gb3, anti-heart, and anti-myosin antibodies. In vitro Gb3 stimulation of patient's peripheral blood mononuclear cells (PBMC) induced high amount production of inflammatory cytokine IL1-β, IL-6, IL-8, and TNF-α. PBMC were stained using the monoclonal antibodies CD3-V500, CD4-V450, CD8-APCcy7, CD45RO-PerCPcy5.5 and CD27-FITC from BD Biosciences and CD56-PC7 from Bekman Coulter. The phenotypic analysis of PBMC showed a lower frequency of CD8 (9.2%) vs. 19.3% and NKT cells (1.6% vs. 2.4%) in Fabry patient respect to healthy donor, suggesting a possible homing to peripheral tissues. A Gb3-induced auto-reactive myocarditis is suggested as a possible cause of FDCM progression and ERT resistance. Immune-mediated inflammation of systemic Fabry cells may coexist and be controlled by implemental immunosuppressive therapy.

Keywords Fabry Disease; cardiomiopathy; inflammation

Received: 8 January 2020; Revised: 13 March 2020; Accepted: 31 March 2020
**Correspondence to: Andrea Frustaci, Department of Cardiovascular, Respiratory, Nephrologic, Anesthesiologic and Geriatric Sciences, La Sapienza University, Viale del Policlinico 155, 00161 Rome, Italy. Email: biocard@inmi.it*

Introduction

Fabry disease (FD) is an X-linked inborn error of glycosphingolipid catabolism caused by deleterious mutations in the GLA (a-galactosidase A) gene encoding the lysosomal hydrolase GAL.[1,2] The marked deficiency or absence of GAL activity results in the systemic accumulation of globotriaosylceramide (Gb3) and related glycosphingolipids within the lysosomes, particularly in microvascular endothelial cells, vascular smooth muscle cells, renal tubular cells, podocytes, and cardiomyocytes.[3–7]

FD cardiomyopathy (FDCM) is a major determinant of patient survival, and its management represents a main therapeutic challenge. Indeed, the impact of enzyme replacement therapy (ERT) on FDCM is still controversial,[8–12] and although there is agreement that early ERT administration, particularly in pre-hypertrophic FDCM, prevents progression of the disease, the advanced form is believed to be irreversible. Mechanisms of resistance to ERT are still unclear, although expansion of interstitial space and myocardial fibrosis appear to be implicated.

To this regard, there is growing evidence that a constitutional secretory pathway of Gb3 from affected cells limits cell engulfment and death, allowing patient survival even in case of absent enzyme activity. Furthermore, there is general agreement on the ability of the extracellular

glycosphingolipids to promote a pro-inflammatory response. A recent report[13] on a large population with FDCM receiving a diagnostic endomyocardial biopsy documents an elevated incidence (56%) of immune-mediated myocarditis reaching the figure of 72% in the cohort with the most advanced phase of the disease (maximal left ventricular wall thickness $>$ 20 mm). These data suggest that a Gb3-induced auto-reactive inflammation of Fabry cells would play a major role in the progression of FDCM as well as in its resistance to ERT. The following study, analysing an explanted heart with FDCM on a 3-year ERT, provides a strong evidence that all affected components of the myocardium including cardiomyocytes, coronary vessels, conduction tissue, and cardiac ganglions can be involved by inflammation causing an incessant electrical instability and the need for cardiac transplantation.

Methods

A severely hypertrophied explanted heart weighting 785 g was examined and processed for histology, electron microscopy, immunohistochemistry, and polymerase chain reaction for viral genomes. In addition, serum samples collected at the time of transplantation were tested for presence of anti-heart, anti-myosin, and anti-Gb3 antibodies.

The explanted heart was transversely cut in sections of 1 cm thick, divided, mapped, and processed in paraffin blocks of 1.5 × 2.5 cm. Paraffin sections of 5 micron were stained with H&E and Masson trichrome. Immunohistochemistry for CD3, CD68, and CD45Ro was obtained for the phenotypic characterization of inflammatory cells. The presence of an inflammatory infiltrate $\geq$14 leukocytes/mm^2 including up to 4 monocytes/mm^2, with the presence of CD3+ T lymphocytes $\geq$7 cells/mm^2 associated with evidence of degeneration and/or necrosis of the adjacent cardiomyocytes, was considered diagnostic for myocarditis. Identification of conduction tissue followed the Aschoff and Monckeberg morphologic criteria and positive immunostaining for HCN4.[14]

For transmission electron microscopy, additional samples were fixed in 2% glutaraldehyde in 0.1 mol/L phosphate buffer (pH 7.3), post fixed in osmium tetroxide, and processed following a standard schedule for embedding in Epon resin. Ultrathin sections were stained with uranyl acetate and lead hydroxide.

Real-time polymerase chain reaction was performed on 5 large tissue samples to search for the most common DNA (adenovirus, cytomegalovirus, parvovirus B19, Epstein–Barr virus, human herpes virus 6, and herpes simplex virus 1 and 2) and RNA (enterovirus, influenza virus A and B, hepatitis C virus) cardiotropic viruses.

Patient serum was tested for the presence of circulating cardiac autoantibodies using a standard indirect immunofluorescence technique.[12]

Patient serum was screened for the presence of antimyosin antibodies, detected by a human myosin ELISA kit (Elabscience Biotechnology Co., Ltd.) and anti-Gb3 Ab ELISA Kit (Biogen scarl-Ariano Irpino, AV) not commercial.

As controls, we used five normal sera from normal subjects matched with patient for age and sex.

PBMC were isolated from whole blood using Ficoll density gradient centrifugation (Cedarlane Laboratories) and cryopreserved in nitrogen liquid. Thawed PBMC were stimulated with Gb3 (1 μg/mL, Matreya LLC) and with SEB (800 ng/mL, SIGMA Aldrich) for 18 h in culture medium (RPMI +10% FBS). IL-1β, IL-6, IL-8, and TNF-α were quantified using ELLA system (ProteinSimple).

PBMC were stained using the following monoclonal antibodies: CD3-V500, CD4-V450, CD8-APCcy7, CD45RO-PerCPcy5.5, and CD27-FITC from BD Biosciences and CD56-PC7 from Bekman Coulter as previously described (Cimini et al., Sci Rep 2017) and acquired to FACS Canto II.

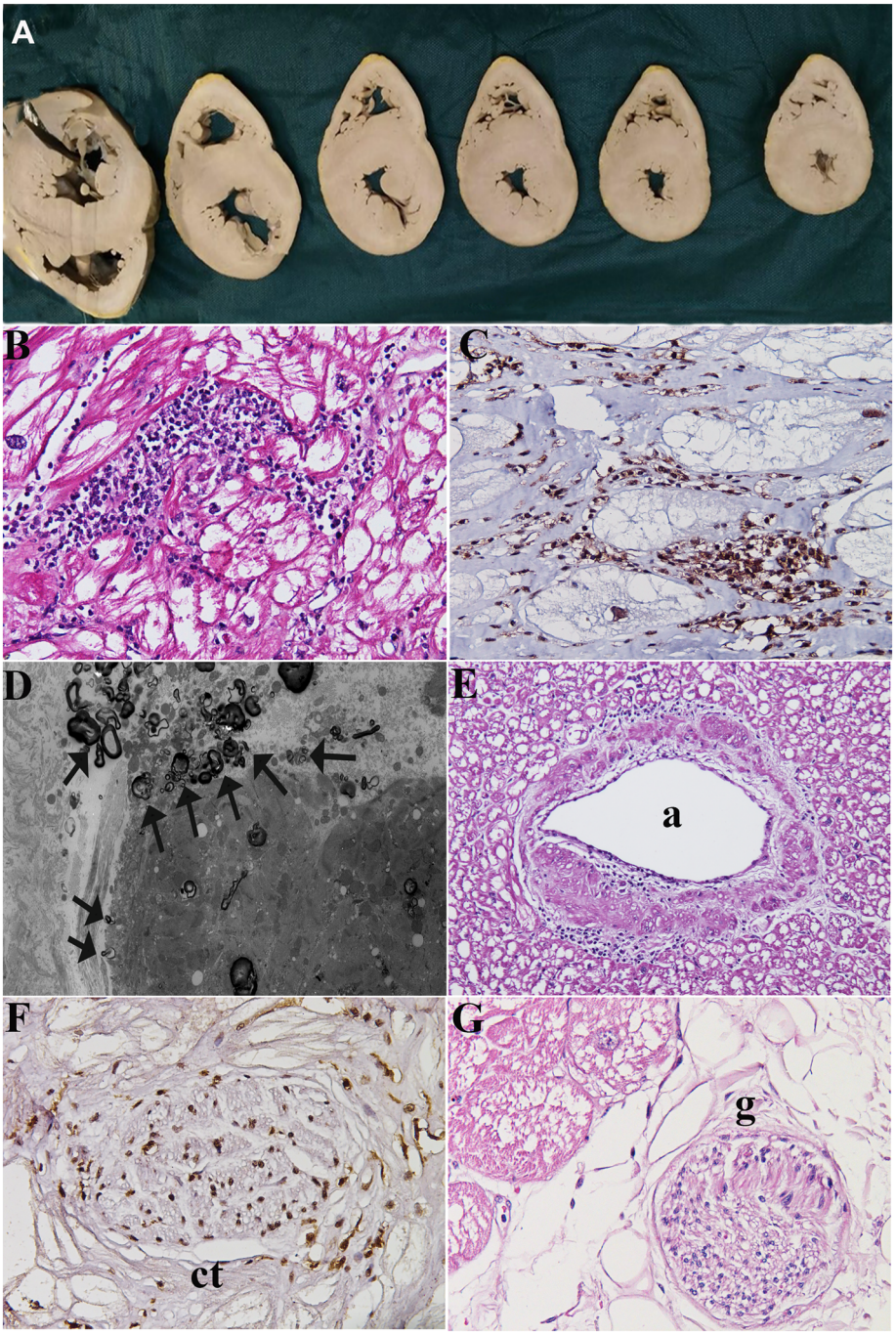
Results

Macroscopic examination (*Figure 1A*) showed a diffuse hypertrophy of cardiac walls with left ventricular maximal wall thickness of 25 mm located at IV septum. Thinning of posterior and lateral LV wall to 7 mm with areas of sclerosis was observed. Epicardial coronary arteries were normal.

Histology, electron-microscopy, and immunohistochemistry

Large (up to 60 μ of transverse diameter at nuclear level), regularly arranged and extremely vacuolated cardiomyocytes were observed (*Figure 1A*). The interstitium was remarkably widened because of fibrosis and extensive inflammatory infiltrates mainly represented by CD3/CD45Ro positive activated T lymphocytes focally associated with necrosis of adjacent myocytes and consisting with severe myocarditis (*Figure 1A,B*). Vacuoles at ultrastructural examination appeared to contain myelin bodies suggesting a Fabry disease with striking intracellular accumulation of glycosphingolipids in the form of myelin bodies and a common evidence of extracellular secretion (*Figure 1C*). Myocardial inflammation was extended to several intramural coronary arteries (vasculitis, *Figure 1D*), to segments of conduction tissue (*Figure 1E*) and to subepicardial ganglia that often showed extensive cytolysis (*Figure 1F*).

Figure 1 Histological and ultrastructural changes of an explanted Fabry heart requiring transplantation because of an incoming ventricular fibrillation. (A) Macroscopic examination of explanted heart showing by sequential cuts severe biventricular hypertrophy. (B) Extensive myocarditis associated to remarkably hypertrophied and vacuolated cardiomyocytes affected by FDCM (H&E 200×). (C) Inflammatory cells being mainly represented by C-activated T lymphocytes (immunohistochemistry with CD45Ro, 200×). (D) Ultrastructural examination showing cardiomyocyte vacuoles consisting of myelin bodies massively secreted (arrows) in the interstitium. (E) Vasculitis of an intramural coronary artery (vessel diameter 180 μm) which wall is infiltrated by glycosphingolipid vacuoles (200×). (F) It shows a section of the vacuolized conduction tissue (ct) infiltrated and damaged by CD45Ro + T lymphocytes (immunohistochemistry 200×). (G) Subepicardial neuron ganglion extensively infiltrated and damaged by T lymphocytes.



DOI: 10.1002/ehf2.12723

Anti-heart, anti-myosin, and anti-Gb3 antibodies

They were found all elevated (*Figure 2*) compared with normal controls.

We therefore tested the ability of Gb3 to induce inflammatory cytokine release by PBMC in Fabry patient and in a healthy control. Results showed that PBMC from Fabry patients respond to Gb3 stimulation producing high amount of inflammatory cytokines (IL1-β, IL-6, IL-8, and TNF-α) (*Figure 2*).

Moreover, the phenotypic analysis of PBMC showed a lower frequency of CD8 (9.2% in PT vs. 19.3% in CTRL) and NKT cells (1.6% in PT vs. 2.4% in CTRL) in Fabry patient respect to healthy donor, suggesting a possible homing to peripheral tissues. Interestingly, both CD8 and NKT cells from Fabry patient showed a skewed differentiation profile with a higher frequency of memory subsets (*Figure* 2I) when compared to a healthy control. In contrast, no differences were observed in CD4 T cells.

Statistical analysis

Data are presented as mean ± SD. A value of $P < 0.05$ was considered as significant. The difference between two groups was determined by unpaired *t*-test for continuous variables.

Discussion

Resistance to ERT is a major therapeutic challenge for patients with FDCM. Indeed, while it is recognized that early ERT administration, particularly in pre-hypertrophic FDCM, prevents progression of the disease, the advanced form is believed to be insensitive/irreversible. Cause of ERT resistance is still unclear although expansion of interstitial space and myocardial fibrosis seem to play a role. To this regard, there is growing evidence that the initial mechanism of interstitial damage might be an immune-mediated myocardial inflammation induced by chronic secretion of the highly immunogenic Gb3 by affected Fabry cells. Gb3 secretion and membrane exposition by cardiomyocytes is a common ultrastructural finding (see *Figure 1C*), particularly in remarkably engulfed cells and explains why FD patients may survive many decades even in the absence of GAL activity. Catabolism of circulating Gb3 into lipoproteins by hepatocytes would concur to the compensatory mechanism.

Recently, myocardial inflammation has been evaluated in a large FDCM population receiving a diagnostic endomyocardial biopsy.[1] An increasing incidence of myocarditis has been documented from 33% of FDCM patients in pre-hypertrophy to 72% of those with advanced (>20 mm of LV maximal wall thickness) disease. Myocardial inflammation was associated with a negative PCR for viral genomes and a positive serology for anti-heart and anti-myosin antibodies suggesting an immune-mediated mechanism.

In the present report, an incoming ventricular fibrillation required a cardiac transplantation in a patient with an advanced FDCM, resistant to ERT administration. Pathology of the explanted heart revealed a severe intracellular accumulation of glycosphingolipids associated to an extensive virus-negative myocarditis. Noteworthy, inflammation involved all affected components of the myocardium including cardiomyocytes, coronary vessels, conduction tissue, and cardiac ganglions. Particularly, inflammations of conduction tissue and of neuron ganglia are unreported in FDCM, because of the difficulty to include them, mostly the latter, in an endomyocardial biopsy, while they played a major role on the electrical instability that required heart replacement.

Diffuse myocardial inflammation in our FDCM patient suggests an auto-reactive myocarditis vs. Gb3 released/membrane exposed by Fabry cells.

This hypothesis is supported by the patient's high serologic titre for anti-Gb3 antibodies, by the inflammatory cytokines released in response to Gb3 stimulation and by the memory phenotype of CD8 and NKT cells.

The initial myocardial damage induced by anti-Gb3 immune reaction would be followed by the release of segregated antigens from insulted myocytes (i.e. myosin) enhancing and perpetuating an autoimmune inflammation (see *Figure 3*). Other organs (kidney and brain) or tissues (vessels) can undergo the same pathway and give a reason for disease progression.

If this hypothesis will be definitely confirmed by further studies, ERT impact on FDCM and on FD in general could be improved by a concomitant administration of low dose immunosuppressive agents.

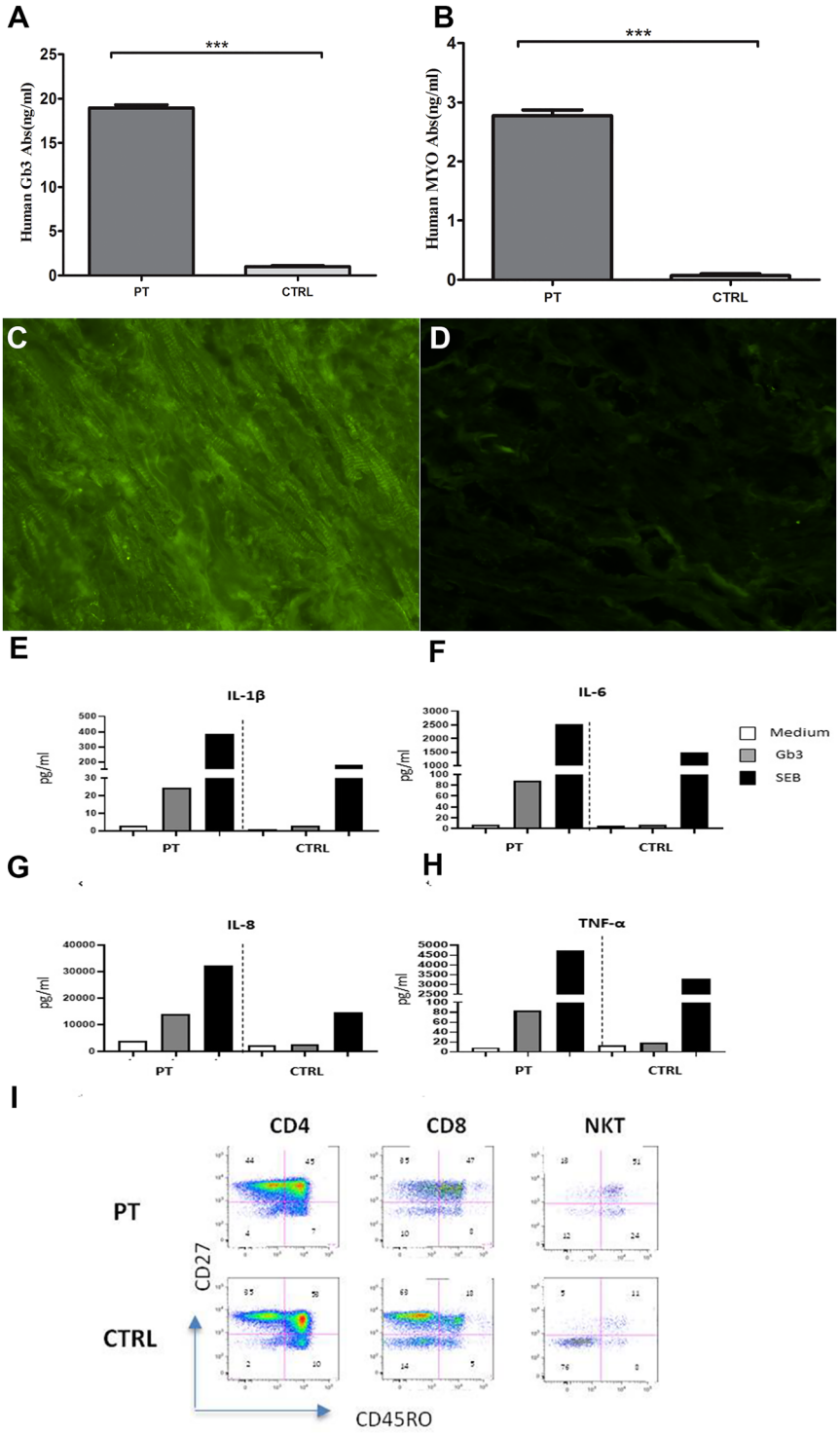
Case report

A 57-year-old male patient was admitted because of cardiac arrest in severe left ventricular hypertrophy.

At the age of 55, he received the diagnosis of Fabry disease (FD) after suffering from a stroke. At diagnosis, he had an a-galactosidase activity of 1.51 nmol/mg/h (n.v. 31.4 ± 12.16), from a missense mutation c.1054G > C (p. A352P). He was receiving ERT (alpha-agalsidase infusion at a dosage of 0.2 mg/kg every other week) since diagnosis up to the admission.

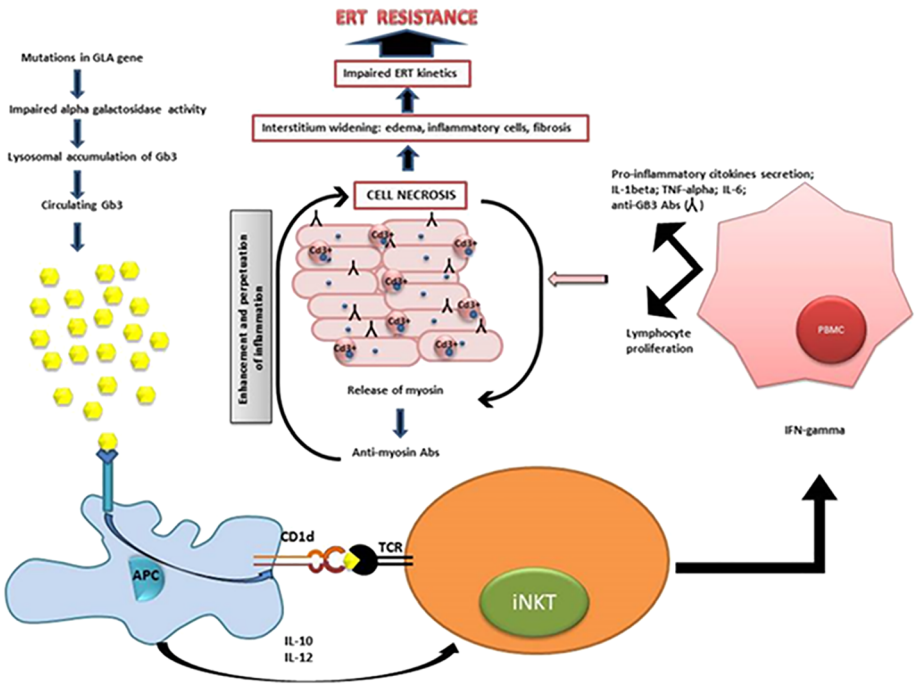
Since he was a child, he was suffering from a peripheral neuropathy, with hypersensitivity to heat and paresthesia, hand burning, bilateral hypoacusia, abdominal pain sometime with diarrhoea. Normal sweating. At the age of 43, he

Figure 2 Profile of anti-Gb3, anti-heart, anti-myosin abs, and PBMC in Fabry patient requiring heart transplantation. (A,B) Graphs show an increase of antibody anti-Gb3(A) and anti-myosin (B) compared with normal controls. (C,D) Anti-heart autoantibodies in Fabry disease patients by indirect immunofluorescence show a strongly positive fine striational pattern of positivity on human heart tissue in sera from patient, compared with control (D) (×400). (E–H) PBMC from healthy control (CTRL) and Fabry patient (PT) were stimulated with Gb3 (1 μg/mL) for 18 h; cytokines (IL-1β, IL-6, IL-8, and TNF-α) were quantified in culture supernatants by Ella system. (I) The differentiation profile of T cells (CD4, CD8) and NKT cells (CD3 + CD56+) were analysed by flow cytometry. CD45RO identifies memory cell subsets: CD45RO + CD27+ cells represent central memory subset while CD45RO + CD27 cells represent effector memory subset.



DOI: 10.1002/ehf2.12723

Figure 3 Represents the hypothesized mechanism underlying autoimmune myocarditis in FDCM. This includes release of Gb3 from Fabry myocytes, antigen exposition by CD1d molecule on dendritic antigen presenting cells which under the action of Toll-like receptor 4 activate the invariant natural killer T cells to a humoral (including anti-glycosphingolipid antibodies) and cellular immune response. Cell necrosis is enhanced and perpetuated by anti-myosin abs.



received a kidney biopsy for chronic renal failure: creatininemia 4.49 mg/dL (n.v. 0.40–1.30), creatinine clearance 17.6 mL/min (n.v. 80–160), proteinuria 3.2 g/die (n.v. 0–200 mg/die). The biopsy was positive for glomerular sclerosis, nefroangiosclerosis with flogistic infiltration and foamy elements of the intimal wall.

The kidney progressively deteriorated, and the patient received a kidney transplant at the age of 52 after 18 months of peritoneal dialysis. After transplant, the renal parameters normalized promptly.

On the neurological side: at age 48, he had the first cerebrovascular MRI due to some declared amnesia events. The MRI described frontal and parietal periventricular gliosis, with vascular space enlargements, with dolico-basilar malformations; at age 55, he had the first stroke which led to the FD diagnosis.

On the cardiac side: at age 47, he suffered from an initial cardiomyopathy and hypertension with an initial concentric hypertrophy which progressively increased to a maximal left ventricular wall thickness of 25 mm. Cardiac MRI with contrast showed a generalized hyperintensity of the signal in the LGE with a generalized intramural involvement. On the cardiac conductance, the patient suffered since 2009 from an atrial fibrillation with complete branch block. Over time, severe arrhythmias brought him to a cardiac arrest at the age of 57 (2019), which led the patient to be a candidate for heart transplantation, performed in March 2019. Waiting for the transplantation, he suffered from 2 further cardiac arrests, 2 further strokes, and he was for 14 days under ECMO. The transplant was successfully performed with no GVH disease.

Conflict of interest

None declared.

Funding

The study has been supported by grants from Telethon (GGP17055), Shire IIR-ITA 002522, Shire International GmbH, and Ricerca corrente IRCCS L Spallanzani, Mebic S Raffaele Pisana.

References

1. Desnick RJ, Ioannou YA, Eng CM. Alpha-galactosidase a deficiency: Fabry disease. In Scriver C. R., Beaudet A. L., Sly W. S., Valle D., eds. *The metabolic and molecular bases of inherited disease*. New York, NY: McGraw-Hall; 2001. p 3733–3774.
2. Desnick RJ. Fabry's disease (aGalactosidase A deficiency): an X-linked nephropathy. In Lifton R., Somlo S., Giebisch G., Seldin D., eds. *Genetic Diseases of the Kidney*. San Diego, CA: Elsevier Academic Press; 2009. p 597–616.
3. Germain DP. Fabry disease. *Orphanet J Rare Dis* 2010; **5**: 1–49.
4. Thurberg BL, Fallon JT, Mitchell R, Aretz T, Gordon RE, O'Callaghan MW. Cardiac microvascular pathology in Fabry disease: evaluation of endomyocardial biopsies before and after enzyme replacement therapy. *Circulation* 2009; **119**: 2561–2567.
5. Najafian B, Savarstad E, Bostad L, Gubler MC, Tondel C, Whitley C, Mauer M. Progressive podocyte injury and globotriasylceramide (GL-3) accumulation in young patients with Fabry disease. *Kidney Int* 2011; **79**: 663–670.
6. Chimenti C, Morgante E, Tanzilli G, Mangieri E, Critelli G, Russo MA, Frustaci A. Angina in Fabry disease reflects coronary small vessel disease. *Circ Heart Fail* 2008; **1**: 161–169.
7. von Scheidt W, Eng CM, Fitzmaurice TF, Erdmann E, Hubner G, Olsen EG, Christomanou H, Kandolf R, Bishop DF, Desnick RJ. An atypical variant of Fabry's disease with manifestations confined to the myocardium. *N Engl J Med* 1991; **324**: 395–399.
8. Shiffmann R, Murray GJ, Treco D, Daniel P, Sellos-Moura M, Quirk JM, Zirzow GC, Borowski M, Loveday K, Anderson T. Infusion of alphagalactosidase A reduces tissue globotriaosylceramide storage in patients with Fabry disease. *Proc Natl Acad Sci U S A* 2000; **97**: 365–370.
9. Schiffmann R, Floeter MK, Dambrosia JM, Gupta S, Moore DF, Sharabi Y, Khurana RK, Brady RO. Enzyme replacement therapy improves peripheralnerve and sweat function in Fabry disease. *Muscle Nerve* 2003; **28**: 703–710.
10. Tondel C, Bostad L, LarsenK K. Agalsidase benefits renal histology in young patients with Fabry disease. *J Am Soc Nephrol* 2013; **24**: 137–148.
11. Rombach SM, Smid BE, Bouwman MG, Linthorst GE, Dijkgraaf MG, Hollak CE. Long term enzyme replacement therapy for Fabry disease: effectiveness on kidney, heart and brain. *Orphanet J Rare Dis* 2013; **8**: 47.
12. Weidemann F, Niemann M, Stork S, Breunig F, Beer M, Sommer C, Herrmann S, Ertl G, Wanner C. Long-term outcome of enzyme replacement therapy in advanced Fabry disease: evidence for disease progression towards serious complications. *J Intern Med* 2013; **274**: 331–341.
13. Frustaci A, Verardo R, Grande C, Galea N, Piselli P, Carbone I, Alfarano M, Russo MA, Chimenti C. Immune-mediated myocarditis in Fabry disease cardiomyopathy. *J Am Heart Assoc* 2018; **7**: e009052.
14. Frustaci A, Verardo R, Grande C, Galea N, Chimenti C. Arrhythmic phenotype of myocarditis sustained by a prominent infiltration of conduction tissue. *Circ Cardiovasc Imaging* 2019; **12**: e009448 Epub 2019 Jul 29.