A pathogenic galactosidase A mutation co-existing with a *MYBPC3* mutation in a female patient with hypertrophic cardiomyopathy

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Title: A pathogenic galactosidase A mutation co-existing with a *MYBPC3* mutation in a female patient with hypertrophic cardiomyopathy

Short title: A *GLA* mutation co-existing with *MYBPC3* mutation

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Brief summary: We reported the co-existence of *GLA* and *MYBPC3* genes mutations leading to a hypertrophic cardiomyopathy in a woman with Anderson-Fabry disease and prevalent cardiac involvement.

Abstract: The co-existence of a *GLA* (Pro259Ser, c.775C>T) and a *MYBPC3* (c.1351+2T>C) mutations was found in a female patient with hypertrophic cardiomyopathy. Histology documented abundant vacuolisation with osmiophilic lamellar bodies and positive Gb3 immunohistochemistry. In the presence of a hypertrophic cardiomyopathy phenotype the systematic search for unusual findings is mandatory in order to rule out a phenocopy.

Case

A 63-years-old Italian woman with history of juvenile asymptomatic paroxysmal Mobitz type I second degree atrio-ventricular (AV) block was admitted to our Hospital for atypical chest pain. Standard 12-lead electrocardiogram showed sinus rhythm with right bundle branch block and negative lateral T waves. Cardiac troponin I variation was not indicative of acute myocardial injury [232-227-224 ng/l; reference range (RR) < 40 ng/l] and coronary arteries were normal at angiography. Echocardiography showed left ventricular (LV) hypertrophy with maximal wall thickness (MWT) of 13 mm at anterior basal septum and LV papillary muscles hypertrophy. Cardiac magnetic resonance (CMR), performed with a 1.5 T magnet (Ingenia, Philips), confirmed LV hypertrophy (LV mass index: 72 g/m^2, RR 52 ± 7.4 g/m^2; MWT at anterior basal septum: 15 mm). Intramyocardial late gadolinium enhancement (LGE) was detected in the basal postero-lateral wall and native T1 was reduced in the postero-basal septum (878 msec; RR: 1003 ± 46 msec) at modified Look-Locker inversion recovery sequence (Fig. 1). Due to the association among hypertrophic cardiomyopathy (HCM), history of AV block and postero-lateral LGE, a genetic analysis was performed, including the following genes: *MYH7, MYBPC3, TNNT2, TNNI3, TPM1, ACTC1, MYL2, MYL3, TTR, GLA, PRKAG2, LAMP2*. Heterozygous mutations in two different genes were found: the splice site mutation NM_000256.3:c.1351+2T>C in *MYBPC3* gene and the missense mutation NM_000169.2:c.775C>T [p.(Pro259Ser)] in *GLA* gene.[1] Plasma Lyso-Gb3 levels were 3.0 ng/ml (RR ≤ 1.8 ng/ml). Dermatological and neurological screenings were negative, renal function was normal except for Maltese cross-like crystals in urinary sediment and cornea verticillata was excluded. Endomyocardial biopsy showed diffuse myocytes hypertrophy with multiple areas of myocardial and myofibrillar disarray and moderate subendocardial interstitial fibrosis. Small arteries with medial hypertrophy and fibrosis of the wall were also present. Marked sarcoplasmic vacuolization consisting of small Periodic acid-Schiff negative/Pearls negative confluent vacuoles was evident in some myocardial areas alongside others without, according to a "patchy" distribution. At ultrastructural examination large vacuoles containing osmiophilic myelinioid lamellar bodies suitable for complex lipid storage were detected (Fig. 2). Enzyme replacement therapy with agalsidase alpha was started and cascade genetic testing performed in all first-degree relatives resulted negative except for the sarcomeric mutation in the son, without clinical expression.

Discussion

Cardiac involvement in Anderson-Fabry (AF) disease is one of the most disabling organ damages, causing HCM, conduction disturbances and arrhythmias. Different missense mutations in *GLA* gene with replacement of the Pro259 amino acid have been reported jet, causing either classical or late-onset phenotype and suggesting a relevant role of this amino acid in the protein function.[2,3] Pro259Ser mutation was reported by Known et al in a 59-year-old man with HCM and perinucleolar vacuolization at endomyocardial biopsy, albeit without ultrastructural characterization.[4] In our case the typical zebra bodies at electron microscopy along with positive Gb3 immunohistochemistry provided evidence of Gb3 storage in myocytes. The patchy distribution of myocyte vacuolization as well as the confined septal low T1 at T1 mapping could be related to the random X-chromosome inactivation in females. Troponin I elevation, already reported in AF disease, could be the expression of chronic cardiac damage in which pro-apoptotic and inflammatory pathways are probably involved.[5]

Regarding the intronic *MYBPC3* variant, *in silico* and *in vivo* specific genetic analyses confirmed its pathogenicity, leading to frameshift change and premature stop codon (Supplemental Methods).

Co-existence of sarcomeric and AF mutations is uncommon but possible in HCM. Differential diagnosis is mandatory since a specific therapy for AF disease is available. Moreover, differences in terms of sudden death risk stratification and genetic counselling of relatives are implied. In the presence of a HCM phenotype, unusual findings like AV blocks, papillary muscles hypertrophy and postero-lateral fibrosis are important red flags that require careful evaluation in order to rule out a phenocopy.

Disclosures

Elena Biagini and Professor Claudio Rapezzi received speaker's fees from Shire and Sanofi-Genzyme. Ferdinando Pasquale received speaker's fees from Shire. The other authors have no conflicts of interest to disclose.

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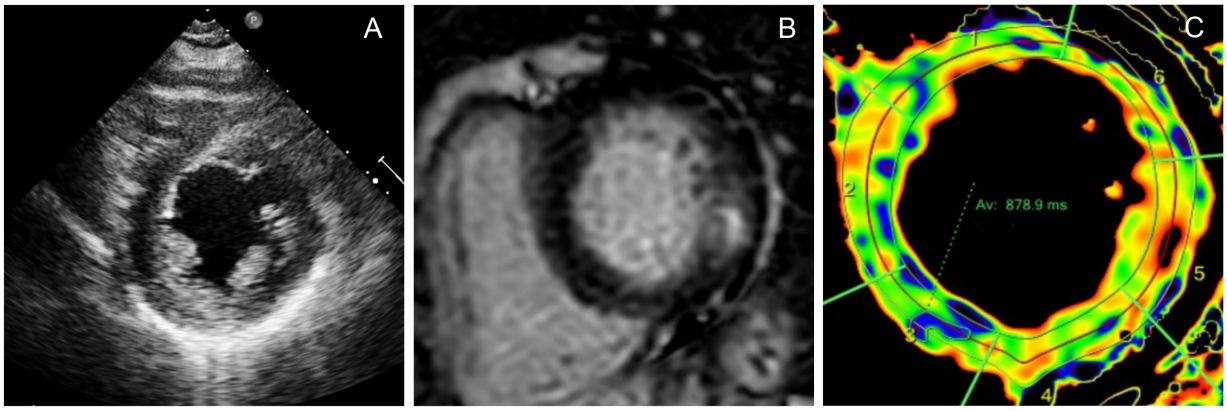
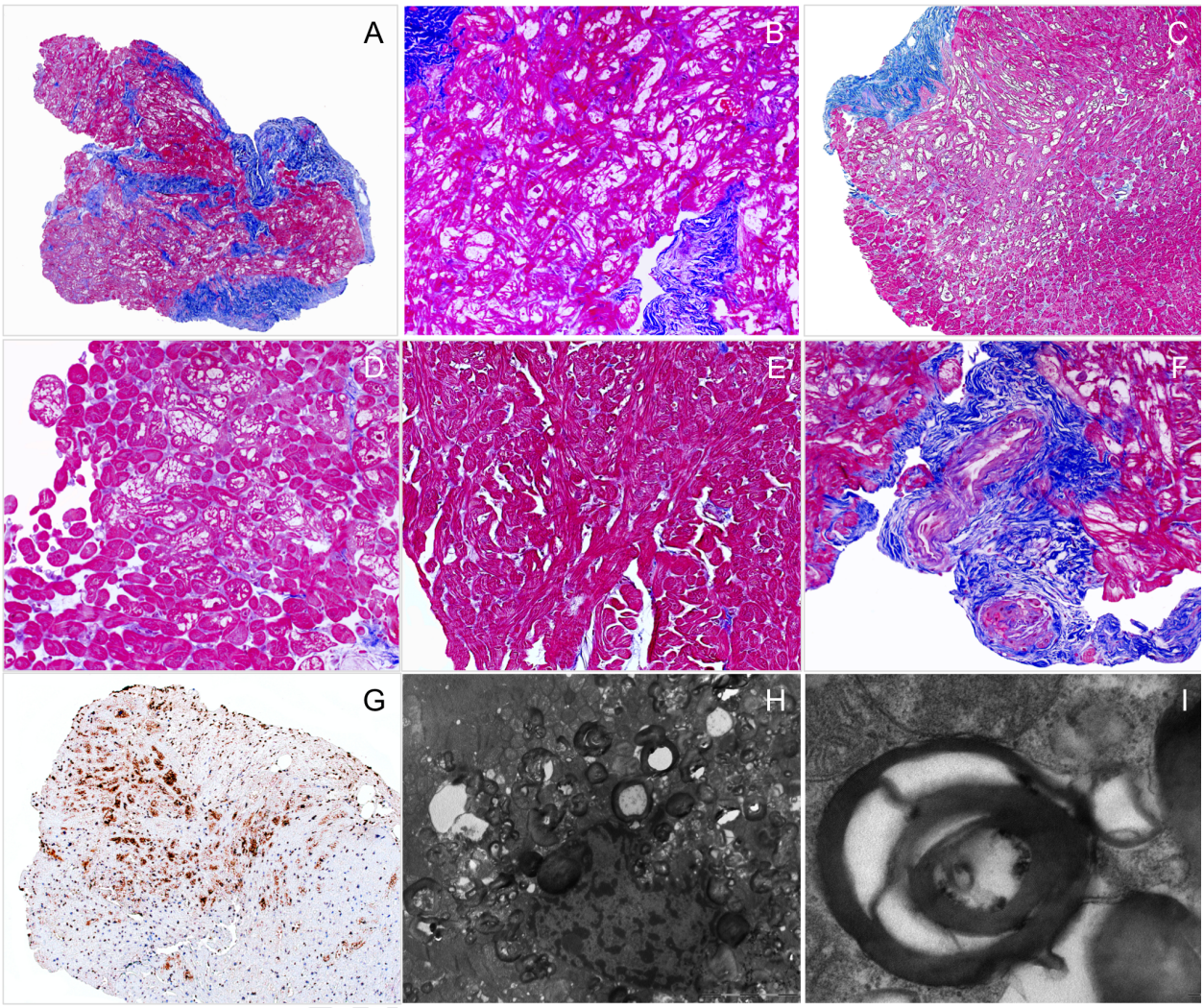
Figure legends:

Figure 1: (A) Echocardiogram showing hypertrophy of papillary muscles. (B-C) Cardiac magnetic resonance with postero-lateral late enhancement (B) and low native T1 at postero-basal septum (C).

Figure 2: (A-F) Histology of endomyocardial biopsy with Mallory trichrome stain showing (A, 50x) moderate myocardial fibrosis, (B, 200x) marked myocyte vacuolization with (C, 100x; D, 200x) patchy distribution, (E, 200x) enlarged myocytes with myocardial disarray and (F, 200x) small vessel disease. (G): Gb3 immunohistochemistry with strong staining of the vacuolated myocytes (100x). (H-I) Ultrastructural features of lysosomal inclusion with a concentric-lamellar pattern.

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