

Case Report

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Decisive role of mRNA sequencing to solve a suspicion of Fabry-disease in a patient with hypertrophic cardiomyopathy and normal GLA DNA sequencing**Albain Chansavang^{1*}; Flavie Ader^{1,2,3*}; Foudil Lamari⁴; Caroline Rooryck⁵; Françoise Pousset⁶; Julie Proukhnitzky^{2,6}; Céline Bordet⁶; Jean-Pierre Rabes⁷; Philippe Charron^{2,6#}; Pascale Richard^{1,2#}**¹APHP, Pitié Salpêtrière- Sorbonne University, DMU BioGem, UF Cardiogenetics and Molecular and Cellular Myogenetics, Department of Metabolic Biochemistry, F-75013 Paris, France.²Sorbonne University, INSERM UMRS 1166 and ICAN Institute, F-75013, Paris, France.³UFR Pharmacy University of Paris Cité, Department 3, F-75006 Paris, France.⁴APHP, Pitié Salpêtrière- Sorbonne University, DMU BioGem, UF Neurometabolism, Department of Metabolic Biochemistry, Paris, F-75013 France.⁵Bordeaux University Hospital, Medical Genetics Department, F-33000 Bordeaux, France.⁶APHP, Reference Center for Hereditary or Rare Heart Diseases, Department of Genetics and Department of Cardiology, Pitié Salpêtrière Hospital, Paris, F-75013 France.⁷APHP, Biochemistry and Genetics Laboratory, Ambroise Paré Hospital, Boulogne, France.

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Abstract

We report the case of a 44-year-old-male presenting with hypertrophic cardiomyopathy and a biochemical analysis suggestive of a Fabry-disease but with a negative coding-regions sequencing of GLA gene (Next Generation Sequencing (NGS), Sanger sequencing and GLA large rearrangement assay). GLA mRNA sequencing was subsequently performed and revealed an abnormal transcript with an intronic sequence insertion due to the deep-intronic hemizygous c.640-801G>A substitution. This deep-intronic region was not sequenced with the next generation custom panel used based on coding regions analysis. In the presence of a clinical suspicion of Fabry-disease and negative results of gene coding regions sequencing, transcript sequencing appears as an appropriate strategy that should be consider solving the diagnostic issue. Following this diagnosis, enzyme replacement therapy has been proposed to the patient, as well as targeted genetic analysis in the family in order to prevent disease progression in relatives.

This case illustrates a particular molecular pitfall regarding the diagnosis of some rare inherited diseases and limitations of NGS/Sanger strategies. Further sequencing mRNA of genes of interest could be a strategy in case of no diagnosis obtained through conventional NGS panel in cardiomyopathies.

Keywords: Fabry disease; RNA sequencing; Cardiomyopathy; lysoGB3.

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Introduction

Fabry disease (OMIM 301500) is an X-linked disorder caused by pathogenic variants in *GLA* gene (NM_000169.3; GenBank accession number NG_007119) leading to a reduction or absence of alpha-galactosidase activity and accumulation of upstream metabolites such as lyso-globotriaosylceramide (lyso-Gb3). In male patients, Fabry disease is divided into “classic” Fabry disease with absent alpha-galactosidase activity, a childhood or adolescent onset and a multiorgan failure at delayed onset, and the “delayed/incomplete” Fabry disease form, characterized by late-onset clinical manifestations during the fourth decade of life including hypertrophic cardiomyopathy and sometimes associated with a decreased glomerular filtration rate, due to residual alpha-galactosidase activity [1].

Confirmatory diagnosis of Fabry disease is usually made by a combination of alpha-galactosidase activity and *GLA* gene disease-causing variant identification, the latter is especially useful for subsequent genetic counseling/testing in relatives and also for the therapeutic discussion since some specific treatments are possible only for particular *GLA* variants [1].

Case presentation

Here, we report the case of a 44-year-old male patient from Cambodia presenting hypertrophic cardiomyopathy (HCM) (Interventricular septum thickness: 30 mm) and slightly reduced glomerular filtration rate (50 mL/min/1.73 m² CKD-EPI formula, and moderate proteinuria 0,11 g/24 hours). No cause of chronic kidney disease at this point has been identified, except history of blood hypertension but well controlled by therapy for many years. No stroke or neuropathic pain were reported. Echocardiography (TTE) revealed Left Ventricular (LV) hypertrophy with maximal wall thickness 30 mm, LV Ejection Fraction (EF) 50% and no outflow tract gradient. The cardiac magnetic resonance imaging (MRI) revealed important late enhancement (diffuse multifocal enhancement >15% LV mass) but without remarkable regional localization and normal T1 mapping relaxation time (1016 ms); LV ED Volume was 105 ml/m², LV EF 60%, LV mass 143 g/m². EKG exhibited a sinus rhythm, PR interval was 190 ms and complete LBBB (QRS duration 140 ms). On holter ECG, non sustained VT as well as few supraventricular bursts were recorded. Cardiac biomarkers were slightly increased at diagnosis (highly sensitive troponine=60 ng/l, normal range <14 ng/l; and NTproBNP 462 pg/mL, normal range <400 pg/mL).

However, leucocyte alpha-galactosidase activity was reduced to 13 nmol/h/mg (normal range 20-80 nmol/h/mg) and lyso-Gb3 was accumulated in plasma (10.1 nmol/L, normal range <0.8 nmol/L) confirmed on several dosages. The patient has signed an informed written consent for genetic analysis. Genetic testing by Sanger sequencing for most frequent hypertrophic cardiomyopathy sarcomeric genes (*MYH7*, *MYBPC3*, *TNNT2*, *TNNI3*), *TTR* and *GLA* failed to detect any pathogenic variant. *GLA* intragenic deletions or duplications were ruled out using by oligonucleotides 60K Custom arrays (Agilent Technologies, one probe every 100 pb), and high throughput sequencing of a panel of 71 genes implicated in cardiomyopathy (including *GLA*) did not detect any pathogenic variant in coding and flanking intronic sequences. After the first negative molecular investigations, further clinical examination did not reveal angiokeratoma

but revealed cornea verticillata. In front of the evocative diagnosis of Fabry disease, analysis of *GLA* mRNA was performed from the patient lymphoblastic cells after reverse transcription and cDNA sequencing. This analysis revealed an abnormal *GLA* transcript containing a 57 bp pseudo-exon inclusion. Genomic DNA sequencing of the corresponding intronic region identified the presence of a hemizygous *GLA* substitution variant NM_000169.3:c.640-801G>A (also named IVS4+919G>A). This variant disrupts a silencer sequence, removing the splicing inhibition resulting in the pseudo-exon inclusion leading to a non-functional protein [2]. Following this diagnosis, enzyme replacement therapy has been proposed to the patient (1.0 mg/kg of Agalsidase alpha). Additionally, targeted genetic analysis have been performed in the family in order to prevent adapt cardiological follow up in the carriers (hemizygous males as well as heterozygous females).

Discussion and conclusion

We report the case of male patient presenting a late-onset Fabry disease with a moderately reduced alpha-galactosidase activity and increased lyso-Gb3 level. Both Sanger and high throughput coding DNA sequencing failed to identify the pathogenic variant but *GLA* transcript analysis revealed the presence of a deep intronic pathogenic variant (NM_000169.3:c.640-801G>A) disrupting normal splicing of *GLA* gene.

This pathogenic variant was previously described in Japanese late-onset Fabry disease male patients presenting 8-15% of residual enzyme activity [2-4] and functional experiments confirmed consequences at protein level [2]. Newborn screening for late-onset Fabry disease variants found that this variant was absent of Korean newborns [5] but highly prevalent in male Taiwanese newborns (1/1460 – 1/875) with <30% of residual enzyme activity [6,7]. The geographic origin of the patient is consistent with the previous cases described. The phenotype associated with this variant in adult male is described to vary from no left ventricular hypertrophy to severe concentric hypertrophy [8], and the expression of the disease didn't seem to be linked to additional pathogenic/likely pathogenic variant [9]. In our patient, the sequencing of coding region of 71 cardiomyopathies genes did not reveal the presence of additional pathogenic/likely pathogenic variant. Familial segregation has also been performed within the family (3 women and 1 man of 50 years old, all asymptomatic with normal cardiological examination EKG and TTE) which is consistent with this phenotypic heterogeneity.

This report first emphasizes the importance of alpha-galactosidase and lyso-Gb3 dosages in front of isolated HCM to properly rule out or suspect Fabry disease in male patients. Second it shows that DNA coding regions sequencing may fail identifying the underlying pathogenic variant especially in case of deep intronic variants. Therefore, a moderately lowered residual enzyme activity observed in a male patient and a negative DNA coding sequencing should not rule out Fabry diagnosis in patients with a clinical/biochemical suspicion of Fabry disease confirming the importance of an accurate clinical diagnosis. Third, this report shows the crucial role of *GLA* transcript (mRNA) analysis in front of a suggestive diagnosis of Fabry disease for subsequent genetic counseling/testing in relatives and

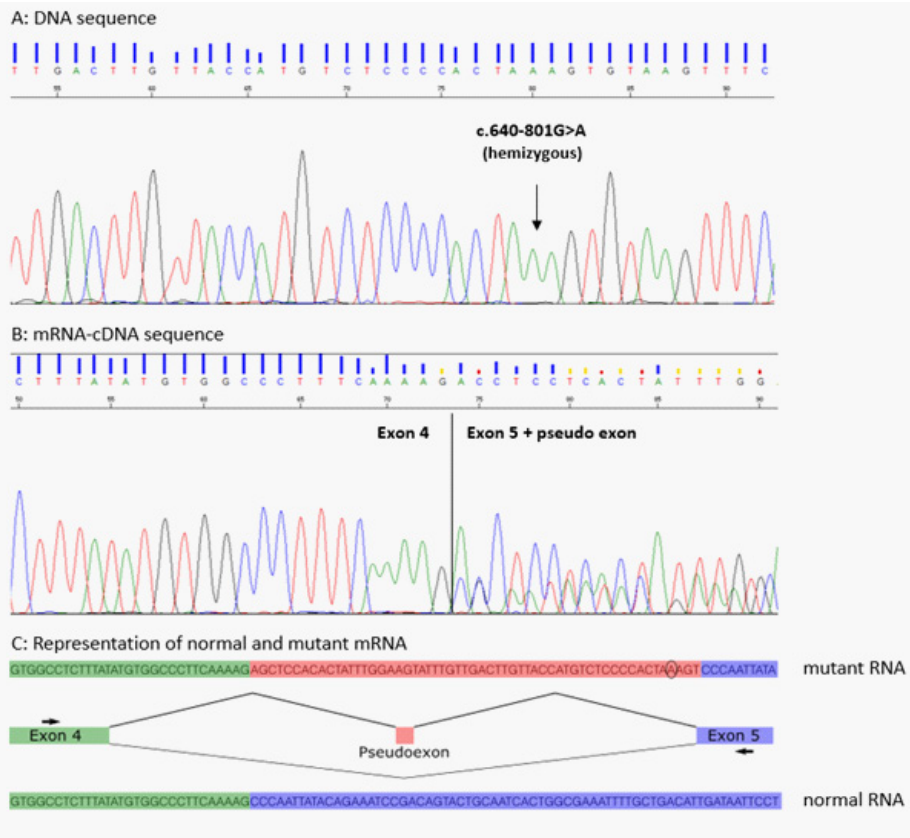


Figure 1: Electropherogram of molecular analyses of the patient and schematic representation of the mechanism leading to the insertion of the pseudo exon in GLA coding sequence.

(A) Sanger sequencing in the proband of the GLA intron 4 region. The GLA genomic variant located position -801 from the exon 5 junction is indicated by an arrow. **(B)** Electropherogram of mRNA sequencing of GLA region (exon 4 and exon 5) including the pseudo exon. The insertion of the pseudo exon is visualized by the superposed sequences. **(C)** Schematic representation of the mechanism leading to the insertion of the pseudo exon: Green rectangle represents exon 4, purple rectangle represents exon 5 and red rectangle represents the intronic regions corresponding to the pseudo-exon.

for the putative therapeutic discussion. Thus, this region should be considered in the NGS panel and the Sanger sequencing on DNA in order to improve efficiency of molecular diagnosis of Fabry cases. In conclusion, the medical community should be aware of such particular molecular pitfalls regarding the diagnosis of rare inherited diseases, and the interest of direct transcript analysis.

Conflict of interest: None declared.

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