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The importance of a multidisciplinary approach in two tricky cases: the perfect match for Fabry disease

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Abstract

Anderson-Fabry disease (AFD) is a multisystem X-linked lysosomal storage disorder caused by a deficiency in the enzyme α-galactosidase A (α-Gal A). This deficiency results in the intracellular accumulation of glycosphingolipids, primarily uncleaved globotriaosylceramide (Gb3) and its deacylated form, lyso-globotriaosylceramide (Lyso-Gb3), leading to progressive organ damage and functional impairment. The diagnostic evaluation for AFD involves clinical assessment and family history, supported by biochemical testing (α-Gal A enzyme activity and Lyso-Gb3 levels) and genetic analysis of the *GLA* gene. In cases of unexplained renal impairment or when genetic analysis is inconclusive, kidney biopsy is often required to confirm the diagnosis and guide targeted treatments. However, histological findings in kidney biopsies may sometimes be nonspecific, complicating the diagnostic process. This article aims to provide an updated perspective on the role of kidney biopsy in AFD, illustrating two cases that exemplify its pivotal role in confirming or excluding the suspected disease, proving to be both decisive and confounding in this complex clinical setting.

Keywords Anderson-Fabry Disease, Kidney biopsy, Zebra bodies, Genetic test

Introduction

Anderson-Fabry disease (AFD) is a progressive lysosomal storage disorder with an X-linked inheritance pattern and a wide range of multisystemic manifestations. It is caused by pathogenic variants in the *GLA* gene, located on chromosome Xq22, which result in varying degrees of α-galactosidase A (α-Gal A) enzyme deficiency and the subsequent progressive accumulation of glycosphingolipids. Globotriaosylceramide (Gb3) and its deacylated form lyso-globotriaosylceramide (Lyso-Gb3) are primarily involved, and they accumulate within multiple cell types, tissues, and organs [1]. Other glycosphingolipid derivatives, such as gangliosides and ceramide-based

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molecules, also contribute to tissue damage and clinical manifestations.

The diagnostic approach for AFD begins with a clinical suspicion based on multi-organ involvement and family history and is confirmed through biochemical, genetic, and histopathological studies. The detection of reduced α-Gal A activity in leukocytes or cultured fibroblasts is the most reliable diagnostic method in males. Elevated plasma Lyso-Gb3 levels can further confirm the diagnosis, particularly in classic patients [1]. In females, diagnosis is more complex because of random X chromosome inactivation, which leads to variability in α-Gal A activity. Variable α-Gal A levels in heterozygous females may overlap with those in unaffected individuals, resulting in diverse phenotypic presentations. In such cases, molecular analysis of the *GLA* gene is mandatory to establish a definitive diagnosis and determine carrier status [2, 3].

Interpreting the pathogenicity of *GLA* variants, particularly variants of uncertain significance (VUS) [4], remains a significant challenge. Kidney biopsy can play a critical role in correlating genotypic findings with phenotypic manifestations. However, reliance solely on pathological findings can sometimes be misleading, as histological changes in AFD may resemble those seen in other conditions. A multidisciplinary approach is essential for accurate diagnosis and effective management of AFD. This paper highlights the role of kidney biopsy in refining the diagnosis of AFD, using illustrative cases to demonstrate its diagnostic value and potential pitfalls.

Cases presentation

Case 1

The first patient was a 55-year-old Caucasian woman presenting clinical features suggestive of multiorgan involvement consistent with AFD. Her past medical history was unremarkable except for minor surgery and hypothyroidism, and routine laboratory analyses did not indicate specific organ dysfunction (Table 1). An ophthalmological evaluation revealed an incidental finding of cornea verticillata, a hallmark feature of AFD. Neurological examination identified widespread sensory abnormalities, including paresthesia in the fingers, tingling sensations in the hands, and hypoidrosis. Biochemical testing revealed reduced α-Gal A enzyme activity in leukocytes and slightly elevated plasma Lyso-Gb3 levels. Genetic testing, including next-generation sequencing (NGS), multiplex ligation-dependent probe amplification (MLPA) and Sanger sequencing of intronic regions, failed to detect pathogenic variants in the *GLA* gene. Cardiological assessments (electrocardiogram, echocardiography, and cardiac MRI) and dermatological evaluations were unremarkable. The patient's adoption precluded access to her family history. However, her 12-year-old son was later recognized with clinical features strongly indicative of familial AFD (Lyso-Gb3: 9.6 ng/mL; enzyme activity: 1.5 nmol/mg/h), confirming the diagnostic hypothesis. She later underwent a renal biopsy to obtain a prompt diagnosis and assess disease staging. Light microscopy revealed diffuse podocyte vacuolization, and electron microscopy demonstrated the presence of Zebra bodies, characteristic of AFD (Figs. 1 and 2). Clinical history did not indicate any other causes of iatrogenic or toxic induced phospholipidosis. Additional findings included mild glomerulosclerosis, interstitial fibrosis, and arteriolar hyalinosis, indicating several chronic changes despite normal albuminuria and proteinuria levels. Histological confirmation prompted further genetic analysis using fibroblast cultures, leading to the identification of a pathogenic *GLA* variant [(NM_000169.2) c.-265G > A]. Further NGS analysis targeted on *GLA* gene and modifications in the interpretation of this variant enabled the confirmation of the mutation even in blood samples, both

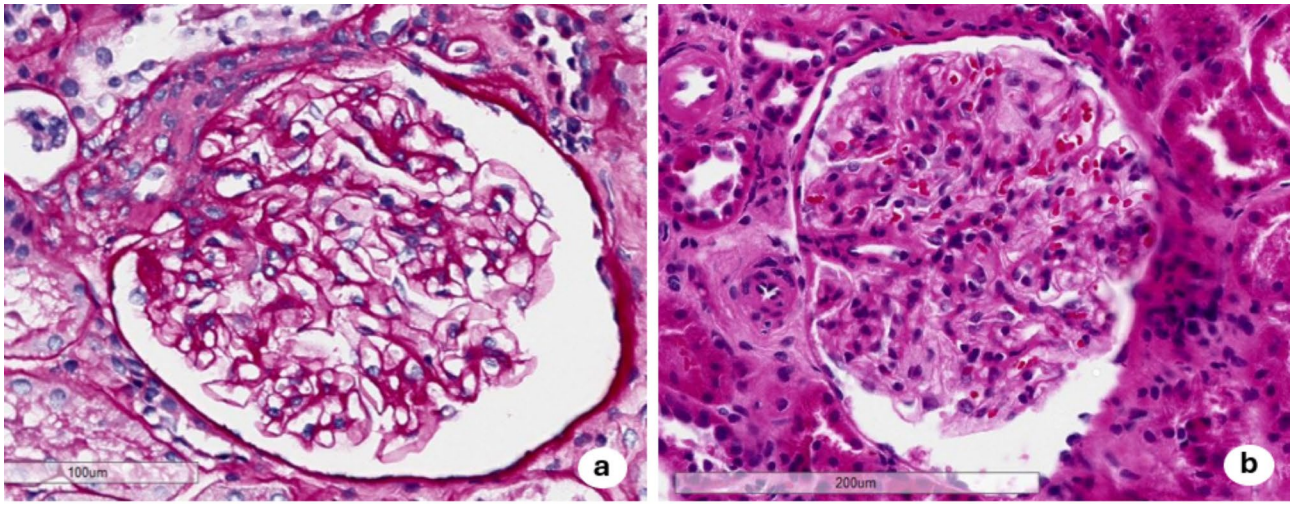


Fig. 1 Light microscopy. Patient 1. Podocytes vacuolization. (**a**) Periodic acid–Schiff stain; (**b**) Hematoxylin and eosin stain

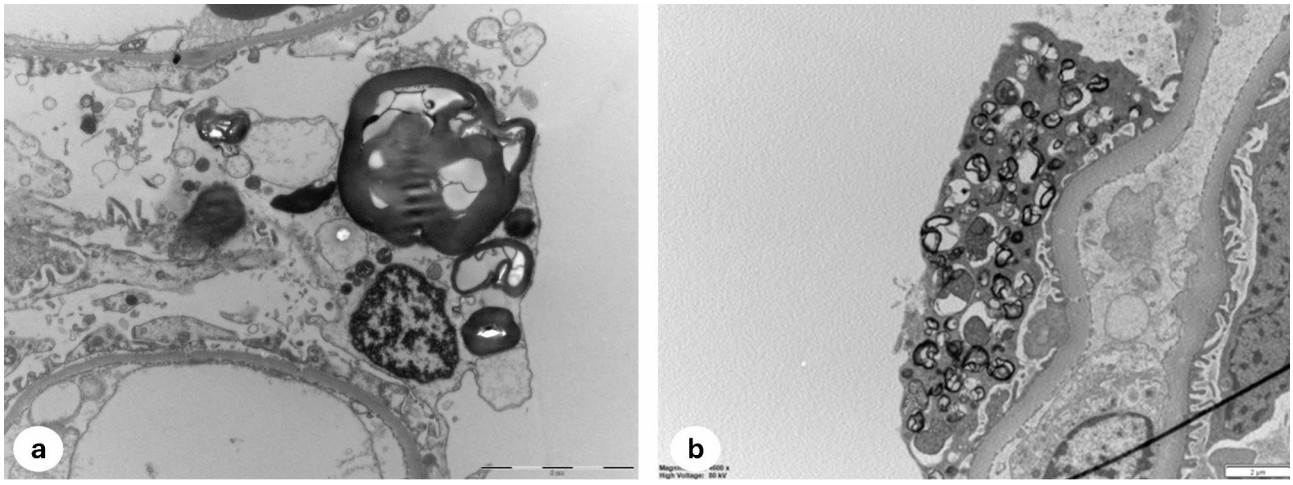


Fig. 2 Electron microscopy. **a**) Patient (1) Zebra bodies in a podocyte (scale bar = 5 µm); **b**) Patient (2) Myeloid bodies in a podocyte

in the patient and her son. This variant is located within a CpG island in the GLA-HNRNPH2 bidirectional promoter. It likely affects *GLA* expression by modifying the promoter's methylation. Based on ACMG/AMP criteria, this variant is classified as a VUS (ACMG 3). Therefore, enzyme replacement therapy (ERT) was initiated.

Case 2

The second patient was a 47-year-old man admitted to the Nephrology Department for progressive proteinuria and chronic kidney disease (CKD) stage 1. His medical and family history were negative for renal diseases. Following the KDIGO 2024 guidelines [5], a kidney biopsy was performed to evaluate the etiology of sub-nephrotic proteinuria in a not hypertensive, young patient. Light microscopy revealed rare podocyte vacuolization, while electron microscopy showed myeloid bodies, which, however, seemed to differ in size and distribution from the classic Zebra bodies typically seen in AFD. These myeloid bodies were smaller and confined to podocytes (Fig. 2). In addition, immunofluorescence analysis confirmed the absence of Gb3 accumulation in renal tissue (Fig. 3).

Both α-Gal A enzyme activity and serum Lyso-Gb3 levels were within normal ranges, and *GLA* gene sequencing, including MLPA analysis, yielded negative results (Table 1). Additional genetic testing for pathogenic variants in *LMX1B*, associated with Nail-Patella syndrome, was negative. A comprehensive evaluation excluded multi-organ involvement typically associated with AFD, including cardiac, dermatological, and ophthalmological manifestations. Drug-induced lamellar deposits and toxic exposures were ruled out. Despite the overlapping histological presentation, diagnosis of AFD was finally excluded and nephroprotective therapy with renin-angiotensin-aldosterone system (RAAS) inhibitors and gliflozins was initiated.

Discussion

Biochemical and molecular analyses are essential to identify cases of AFD, but sometimes the results can be inconclusive, prompting the use of histological evaluations to help the diagnosis. Kidney biopsy is a cornerstone in nephrology for diagnosing renal diseases and guiding treatment decisions. With advancements in radiological guidance, renal biopsy has become a safe and routine procedure [6]. Although genetic testing is widely available for the diagnosis of AFD, kidney biopsy remains a valuable tool, especially in complex genetic cases, such as variants of unknown significance (VUS) [7] or non-coding *GLA* variants that are not routinely included in standard diagnostic protocols. Recent studies [8, 9] have examined the actual pathogenic effects of non-coding mutations, demonstrating that they can have an impact similar to pathogenic variants occurring in coding regions. These mutations may directly alter the enzyme's peptide sequence, structure, and function, thereby contributing to disease pathogenesis. However, even in these cases the utility of kidney biopsies continues to be a subject of debate [10].

Histological findings, as widely described in the literature, provide valuable insights into the severity of renal involvement, often revealing subclinical damage and response to treatment [11, 12]. Studies by the International Study Group of Fabry Nephropathy (ISGFN) underscore the utility of kidney biopsy as part of the baseline assessment of the AFD nephropathy [13]. The real chronicity status pointed out by glomerulosclerosis, interstitial fibrosis and arteriolar hyalinosis, was detected even in patients at incipient stages with minimal or absent proteinuria. These findings suggest that histological renal involvement precedes overt clinical signs, highlighting the value of early detection.

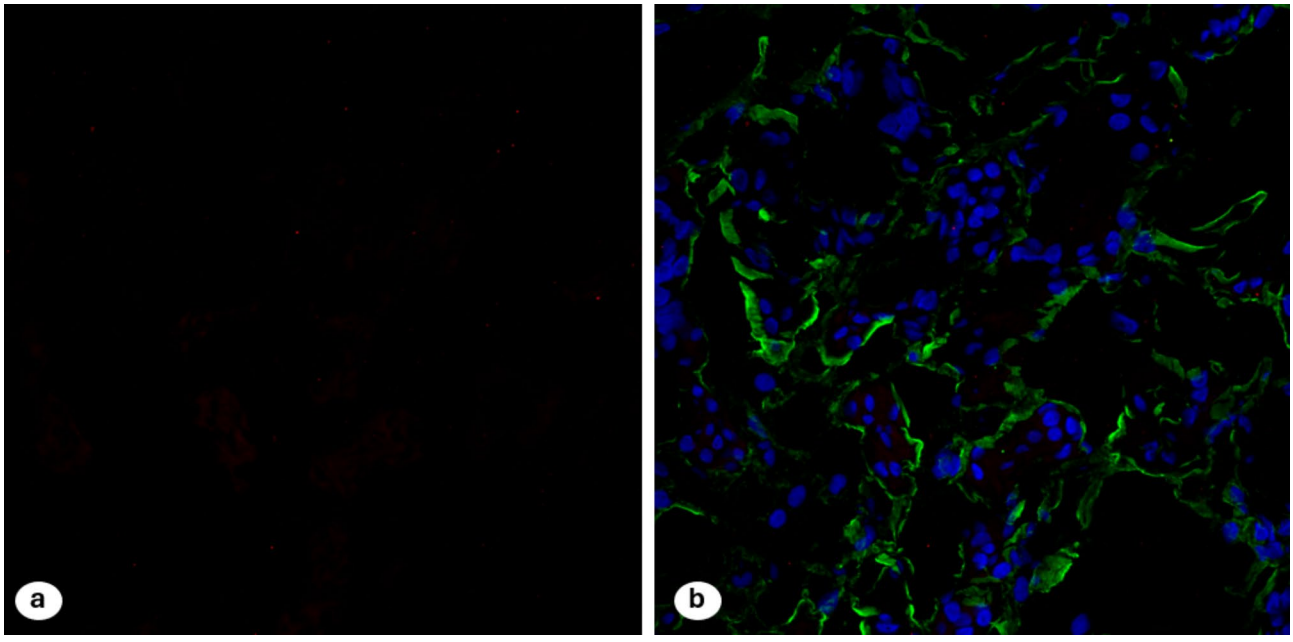


Fig. 3 Negative Gb3 immunofluorescence performed on renal sample of Patient 2. (**a**) Negative Gb3 (red); (**b**) Merge of negative Gb3 (red), positive collagen (green), and nuclei (blue). 50-µm sections were prepared using a freezing sliding microtome (HM550, Thermo Scientific, Waltham, MA, USA) to evaluate Gb3 deposits. The sections were immunostained overnight with a panel of primary antibodies, including mouse monoclonal anti-Gb3 (1:1000; TCI Chemicals, Portland, Oregon, USA), rabbit pan-neuronal marker protein gene product 9.5 (1:1000; AbD Serotec, Raleigh, NC, USA) and rabbit polyclonal anti-collagen IV (1:500; Novus Biologicals, Littleton, CO, USA). After washing, secondary antibodies were applied for an additional overnight incubation. Mouse cyanine dye fluorophores 3.18 (1:800; Jackson ImmunoResearch, West Grove, PA, USA) were used as secondary antibodies. Sections were viewed and analysed under a Nikon confocal microscopy (Eclipse Ti A1)

All observed structures exhibit lipid inclusions and show early stages of sclerosis, even in patients with non-classical mutations and VUS [14]. Fibrosis, as observed in other chronic diseases, represents the final stage of tissue damage; however, few studies have investigated the pathogenesis of fibrosis in AFD nephropathy. The deposition of Gb3 in various renal cell types triggers biological processes that induce the production of pro-inflammatory cytokines and promote fibroblast proliferation [15]. At present, kidney biopsy remains the only reliable method to assess the degree of kidney fibrosis, unlike other organs such as the heart and brain, where innovative imaging techniques provide sensitive evaluations of fibrosis. It is well-established that fibrosis can limit the therapeutic response to ERT. Therefore, a deeper understanding of the molecular mechanisms

Table 1 Demographic and laboratory data of the two case reports

	Patient 1	Patient 2
Gender	Female	Male
Age, years	55	47
Systolic/diastolic blood pressure, mmHg	120/85	125/80
Hemoglobin, g/dL	13	16.9
Serum creatinine, mg/dL	0.79	0.9
eGFR, mL/min/1.73m^2	74	106
Proteinuria U 24 h, mg/day	110	2300
Na/K, mmol/L	141/3.7	140/4.4
Lyso Gb3, ng/mL (cut-off < 1.8)	2.5	< 1.8
Enzyme α -galactosidase A activity nmol/mg/h (n.v. 20–65)	7.8	65
GLA exon's sequencing and MLPA	Negative*	Negative
Troponin I, ng/L (cut-off < 19.8)	4.8	5.7
BNP, pg/mL (cut-off < 100)	13	55
C3/C4, mg/dL (n.v. 90–180/10–40)	142/35	132/37

eGFR, estimated glomerular filtration rate. BNP, brain natriuretic peptide. C, complement. n.v., normal value. *At initial evaluation

Note: Conversion factors for units: serum creatinine in mg/dL to µmol/L, ×88.4

underlying fibrosis may lead to the development of new therapeutic strategies [16]. Additionally, confocal microscopy studies using fluorescent mouse monoclonal anti-Gb3 antibodies can be applied to biopsy specimens to confirm Gb3 deposits in ambiguous cases [17].

Regarding the prognostic value of kidney biopsy, recent studies by Michael Mauer and Behzad Najafian have focused on podocyte involvement in the progression of AFD nephropathy. By leveraging ultrastructural morphometric measurements of Gb3 inclusions, their research developed a quantification method to describe the percentage of podocytes exhibiting the AFD phenotype. This method is adjusted for age and sex while addressing the heterogeneity of podocyte involvement in female mosacism [18–20]. Future research goals include establishing a rigorous histology-based risk stratification model for disease progression and identifying a hypothetical threshold for podocyte Gb3 inclusion volume to mitigate glomerular stress through early treatment.

Lastly, kidney biopsy provides distinctive features that can aid in differentiating superimposed renal diseases, especially when the clinical context, genetic data, and family history are unclear [21].

The two cases presented here illustrate the role of kidney biopsy in resolving diagnostic challenges in AFD and demonstrate its critical role when clinical and laboratory findings alone are insufficient for a definitive diagnosis. Our experience emphasizes the significance of kidney biopsy in cases with complex genetic backgrounds (patient 1), while highlighting the importance of a multidisciplinary approach in addressing the diagnostic challenges posed by overlapping histological features (patient 2).

In the first case, kidney biopsy played a pivotal role in clarifying the diagnosis despite discrepancies between clinical and laboratory findings. The histological suspicion prompted further diagnostic evaluation, including genetic analysis of fibroblast cultures, which ultimately led to a precise diagnosis of AFD. A key lesson from this case is that in patients with a clinical phenotype consistent with AFD, but no identified *GLA* gene variant, it is important to thoroughly review genotyping data and consider alternative molecular diagnostic methods.

On the other hand, the second case highlights the limitations of relying solely on histological findings for diagnosis and underscores the importance of a multidisciplinary approach that integrates genetic testing, second level histological analysis, and comprehensive clinical and laboratory evaluations to rule out misleading diagnoses. In this patient, the pathology report alone was confusing and confirmed the value of enzymatic and genetic testing.

Histological features should always be interpreted in conjunction with clinical data in a multidisciplinary setting. Myeloid bodies, indeed, are not an uncommon as ultrastructural finding in kidney biopsies, and they can also be found in patients without clinical evidence of AFD [22, 23]. Therefore, further work-up is necessary to exclude drug- induced phospholipidosis (DIP), toxins, and other inherited diseases such as Nail Patella Syndrome or Niemann-Pick disease [24]. Despite overlapping histological features, distinguishing between AFD and these other conditions is possible. In non-AFD kidney biopsies, myeloid bodies are focal and predominantly localized within podocytes. Furthermore, the inclusions tend to be smaller, scattered, and may completely resolve upon withdrawal of the inciting agent. In contrast, AFD kidney biopsies exhibit permanent lamellar inclusions, generally known as Zebra bodies, present in at least two distinct cell types: podocytes, parietal cells, distal tubular epithelium, and endothelial cells [24]. To better differentiate between these two entities, we propose that Zebra bodies should be considered highly diagnostic of AFD, whereas the presence of nonspecific Myeloid bodies requires further context to assess their clinical relevance.

This analysis confirms the importance of a histological evaluation conducted by expert pathologists, in collaboration with clinical teams, to accurately characterize the nature of deposits and resolve diagnostic dilemmas. In rare diseases like AFD, an integrated, multidisciplinary approach is crucial for achieving an accurate diagnosis and avoiding misdiagnosis or delays. Podocyte vacuolization and intracellular lamellar inclusions observed on electron microscopy, in the context of other characteristic features, may confirm the diagnosis of this lysosomal storage disorder, but they can also be secondary to other conditions. Following these considerations, the essential role of histological evaluation clearly comes into light as both a decisive tool and a potential source of diagnostic confusion in the complex clinical setting of AFD. Prompt recognition of these challenges and the implementation of a comprehensive evaluation will help ensure optimal patient outcomes.

Abbreviations

AFD	Anderson-Fabry disease
ERT	Enzyme replacement therapy
CKD	Chronic kidney disease
ISGFN	International study group of Fabry nephropathy
DIP	Drug- induced phospholipidosis

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Not applicable.

Author contributions

Conceptualization: [GMB, VA, GV, IC, RM, GLM]; Methodology: [GMB, GV, BF, IC]; Formal analysis and investigation: [GMB, VA, SL, FC, MS, VD, EB, MF, FM]; Writing - original draft preparation: [GMB]; Writing - review and editing: [GV, FC, VD, EB, MF, BF, FM, DT, GP, RM, GLM, IC]; Funding acquisition: [N/A]; Supervision: [GV, DT, GP, RM, GLM, IC]

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No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate
The study was performed according to the Declaration of Helsinki.

Consent for publication
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Competing interests
The authors declare no competing interests.

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